Accumulation of \(^{3}H\)Glycine by Cone Bipolar Neurons in the Cat Retina

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ABSTRACT
Cone bipolar neurons in the cat retina were studied in serial sections prepared as electron microscope autoradiograms following intravitreal injection of \(^{3}H\)glycine. The goal was to learn whether the cone bipolar types that accumulate glycine correspond to the types thought on other grounds to be inhibitory. About half of the cone bipolars in a given patch of retina showed specific accumulation of silver grains. The specificity of accumulation was similar to that shown by glycine-accumulating amacrine cells. All of the cone bipolars arborizing in sublamina \(b\) accumulated glycine but none of the cone bipolars arborizing in sublamina \(a\) did so. The types of cone bipolars accumulating glycine did not match the types thought to be inhibitory. Cone bipolar types \(C_{B}B_{1}\) and \(C_{B}B_{2}\) both form gap junctions with the glycine-accumulating \(A_{II}\) amacrine, thus raising the possibility that glycine might accumulate in these cone bipolars by diffusion from the \(A_{II}\) cell or vice versa. Thus it is logically impossible to tell which of these three cell types contains a high-affinity uptake mechanism for glycine and consequently which of the three might actually use glycine as a neurotransmitter.

Key words: All amacrine, gap junction, uptake, neurotransmitter, autoradiography

In the vertebrate retina the amino acid glycine satisfies several of the fundamental criteria for an inhibitory neurotransmitter. Application of glycine by superfusion or iontophoresis suppresses the firing of ganglion cells (Boltz et al., '85; Saito, '80) and hyperpolarizes ganglion, amacrine, and bipolar cells by increasing conductance to chloride ions (Miller et al., '81a, b). These effects are antagonized in a specific manner by strychnine (Boltz et al., '85; Saito, '80), long known as a blocker by synaptic inhibition (Sherrington, '06). Glycine is naturally present in the retina (Pawant-Morales et al., '72; Cohen et al., '73) and can be released by light stimuli or by depolarizing synapses with potassium (Ehinger and Lindberg-Bauer, '76). Finally, there is a high-affinity uptake mechanism for glycine, and this could serve to remove the compound from the synaptic cleft following its release (Voeden et al., '78; Chin and Lam, '80).

The question of which cell type uses glycine as a transmitter is still unanswered. No specific synthetic enzyme has been identified to serve as a marker as in the cases of other transmitter systems such as acetylcholine, GABA, and dopamine. Therefore, neurons that might release glycine have been identified only by accumulation of silver grains over their cell bodies in autoradiograms following the administration of exogenous \(^{3}H\)glycine. Several types of amacrine and also certain cone bipolar neurons have been identified by this method (e.g., Pouzo, '80; Marc, '81; Frederick et al., '84). The autoradiographic pattern appears to represent the accumulation of glycine rather than its conversion into other small molecules or its incorporation into protein. This is indicated by the observation that more than 90% of the injected radioactivity remaining in the tissue after 1 hour comigrates in chromatograms with authentic glycine (Ehinger and Patel, '71; Voeden and Marshall, '74; Freed and Sterling, unpublished). Unfortunately, accumulation of glycine by a neuron does not, necessarily indicate that it is released by that neuron. It is conceivable, for example, that at a glycineergic junction the postsynaptic neuron rather than the presynaptic neuron is the glycine-accumulating.

One way to explore this problem further would be to determine whether glycine is accumulated by neurons that are thought on other grounds to be inhibitory. The \(A_{II}\) and \(A_{III}\) ganglion cells in cat retina are each innervated by a pair of cone bipolar neurons, and one member of each pair

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is thought to be inhibitory (Fig. 1; McGuire et al., '86). The All amacrines cell is also thought to be inhibitory to the eff-
beta ganglion cell both pre- and postsynaptically (Sterling, '83). If glycine is the transmitters, any of these putative inhibitory synapses, and if glycine accumulation is a rela-
tively marker of this function, one would predict accumulation by cone bipolar types CBa, CBb, and by the All amacrine. Cone bipolar types CBa, CBb, thought to be excitatory (Sterling, '83; McGuire et al., '86) should not accumulate glycine. This prediction was explored in the present study.

METHODS

Three cats were used for this study. Each cat was anesthe-
tized with Nembutal (40 mg/kg) and one eye was injected intravitreally with (H)glycine (25--100 μg/mL 44-64 Cusum) in 25--100 μL of saline solution near the retina, causing which was identified ophthalmoscopically. In one of these experiments the glycine (23 μM) was injected in a buffered mixture of 5 mM sodium, 5 mM proline, and 5 mM serine in order to block low-affinity uptake of neutral amino acids (Chin and Lamm, '80). After 1 hour, the cat was perfused intracardially with 2% paraformaldehyde-glutaraldehyde mixture in 0.12 M phosphate buffer.

The retina was osmicated, stained en bloc with uranyl acetate, dehydrated, and embedded in Epon. For light mi-
croscopy, we cut 1-μm-thick sections from the retina, coated them with Kodak NTB3 emulsion, and exposed for 18 days. The autoradiograms were developed in D-19 (2 minutes at 19°C), fixed, and stained with quinacrine blue. For electron microscopy we cut 270-μm-thick sections (state gold color) and processed them in the electron microscope (EM) au-
toradiography by using Iodol E4 emulsion according to Davis et al. ('79). After a month's exposure the sections were developed in Kodak HC10 for 2 minutes, fixed, and stained with uranyl acetate and lead citrate. Sections were photographed on a JEM 1200 electron microscope at 1.7000V at 120 K. Cone bipolar axons were reconstructed by tracing their outlines in successive sections onto acetate sheets that were aligned on a carbon-based jig. The re-
constructed axons were displayed by digitizing the traces into a computer with a graphics tablet. Grain densities of gly-
cine-accumulating profiles in EM autoradiograms were de-
termined by using a graphics tablet to measure profile area and were averaged over a minimum of ten successive sections.

RESULTS

Overall distribution of silver grains

The general distribution of silver grains at the light mi-
icroscopic level, following topiocular injection of tritiated glycine has been studied before in this and other laborato-
ries (Nakamura et al., '78; Poucet '80; Shingler and Fain, '83), and the present results confirm previous observations (Fig. 3). Thus, there were few silver grains over the photo-
receptor outer segments, outer nuclear layer, outer plex-
iform layer, and horizontal cells. There were moderate accumulations in the inner nuclear layer over Müller cell bodies and strong accumulations over certain cone bipolar and amacrine cell bodies. Many grains were present in the inner plexiform layer, concentrated mainly over strata 2-
4. Moderate accumulations were present over ganglion cell bodies of all sizes, and very dense accumulations were pres-
**GLYCINE-ACCUMULATING CONE BIPOLARS**

Fig. 2. Light micrograph autoradiogram showing distribution of silver grains across the retina. In the inner nuclear layer (INL) certain cone bipolar cells (arrowed and arrowed) bear many silver grains; other cone bipolar terminals and amacrine bear few. InL, inner plexiform layer; GCL, ganglion cell layer.

Fig. 3. EM autoradiogram. An amacrine in the ganglion cell layer bears many silver grains. The cell forms processes that ensheath ganglion cell axons. This cell may correspond to the amacrine observed by Freed et al. (1981) that accumulates GABA.

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**Fig. 1.** Identified pathways in cat retina. Arrow (black) presumed excitatory connections. Arrow (white) presumed inhibitory connections. Arrow (grey) presumed unidirectional or reciprocal connection. INL, inner nuclear layer; IPL, inner plexiform layer; AII, OFF-Beta, ON-Beta.
Fig. 4. EM autoradiogram of the inner nuclear layer (INL). The somas of certain cone bipolar (CBb) and amacrine (AM) keep many silver grains. The somas of other cone bipolar (CBB), rod bipolar (RB), and horizontal cells (HC) have few grains. OPL, outer plexiform layer; IPL, inner plexiform layer.

![Autoradiogram of the inner nuclear layer (INL).](image)

Fig. 5. Histogram showing accumulation of grains by the major cell groups in the inner nuclear layer. Note that horizontal, Müller, glial, and certain bipolar and amacrine somas accumulate few grains. Certain amacrine and cone bipolar neurons accumulate many grains.

![Histogram showing accumulation of grains.](image)

| TABLE 1. (14C Glycine Accumulation by Cell Classes in the Inner Nuclear Layer) |
|---------------------------------|----------------|
|                                | Experiment 1 | Experiment 2 |
|                                | No. of cells | Mean ± S.D. | No. of cells | Mean ± S.D. |
| Cones bipolar                   |              |              |              |
| Unlabeled                       | 17           | 66 ± 1.3     | 4            | 326 ± 3.6    |
| Labeled                         | 24           | 34 ± 5.9     | 3            | 166 ± 5.2    |
| Amacrine cells                  |              |              |              |
| All amacrine cells              | 53           | 66 ± 3.1     | 7            | 326 ± 1.0    |
| Labeled                         | 14           | 40 ± 1.0     | 15           | 160 ± 1.0    |
| Horizontal                      |              |              |              |
| All horizontal cells            | 6            | 46 ± 1.5     | 4            | 120 ± 1.5    |
| Labeled                         | 10           | 46 ± 1.5     | 7            | 120 ± 1.5    |
| Müller elements                 | 6            | 34 ± 1.5     | 7            | 34 ± 1.5     |
| Other                           | 1            | 11 ± 1.1     |              |              |

These results strongly suggested that all cone bipolar arborizing in sublamina b are labeled by exogenous glycine and that all cone bipolar arborizing in sublamina a are unlabeled. Nevertheless, it was important to check by direct observation whether the type CBb cone bipolar, thought to be excitatory in sublamina b, was actually labeled or whether it had somehow been omitted from our sample. We located three labeled axons thought to be of this type and reconstructed them in order to establish their identities.
Fig. 6. Tangential view of the inner nuclear layer showing even distribution of labeled and unlabeled cone bipolar axons.

Fig. 7. Reconstructions from 279 serial sections of three type CBIb axons. The thick axon stalk descends into the inner plexiform layer (IPL) to sublamina B where it forms a narrow-field classlike arborization. Each axon has five or nine gap junctions (parallel lines) with all amacrines cells. INL, inner nuclear layer; GCL, ganglion cell layer.
Fig. 8. Large gap junction (thick arrow) between type CB\(\text{B}_{\text{b}}\) cone bipolar and an All amacrine. The CB\(\text{B}_{\text{b}}\) forms a ribbon synapse (thin arrow). NAD silver grains in both CB\(\text{B}_{\text{b}}\) and All processes.

Fig. 9. Gap junction from Figure 8 enlarged to show detail. The plasma membranes of the CB\(\text{B}_{\text{b}}\) and All are closely apposed, utilizing the extra cellular space.

Each had a thick stalk descending its sublamine 6 where it formed a clawlike fiber in strata 3-5 (Fig. 7). Each arborization formed multiple, large gap junctions with the type All amacrine (Figs. 8, 9). These reconstructed amacrine types correspond exactly to the one bipolar type called CB\(\text{B}_{\text{b}}\) by McGuire et al. (94) and therefore demonstrate that this type accumulates excessive glycine.

DISCUSSION

The question studied here was whether glycine is accumulated by retinal neurons thought on other grounds to be inhibitory and not by neurons thought to be excitatory. The answer is yes. The All amacrine, whose chemical synaptic output in sublaminas A is thought to be inhibitory (Sterling, 53), does accumulate glycine (see also Pourcho, 52, Pourcho and Geese, 53), and this is consistent with its being glycogenic. None of the cone bipolar cells arborizing in sublaminas A accumulates \(\gamma\)-Glycine. Therefore, the type CB\(\text{B}_{\text{b}}\) cone bipolar (Fig. 1), if it is indeed inhibitory, probably uses some other transmitter. All the cone bipolar neurons in sublaminas B accumulate glycine. Therefore, type CB\(\text{B}_{\text{a}}\), which is thought to be inhibitory, might be glycogenic. Doubt is raised, however, by the fact that type CB\(\text{B}_{\text{a}}\), which is almost certainly excitatory (McGuire et al., 96), also accumulates glycine. Thus it is unlikely that the glycine accumulated by CB\(\text{B}_{\text{a}}\) is released by it as an inhibitory transmitter. These results cast doubt on the reliability of this approach in identifying glycogenic receptors.

The present results raise an additional and even more basic question: which cell types contain the high-affinity uptake mechanism for glycine? A neuron may accumulate glycine by means of its own high-affinity uptake mechanism or, since glycine is a small molecule, by diffusion from other neurons to which it is connected by gap junctions. The All amacrine is connected to the type CB\(\text{B}_{\text{b}}\) cone bipolar by numerous, large gap junctions and also to the other types of cone bipolar in sublaminas B by long numerous and smaller gap junctions (McGuire et al., 94). The present observations offer no way to determine whether the high-affinity uptake mechanism is located in the All cell or to one or more types of cone bipolar. The general caution emerges from these experiments that one cannot reasonably assign a high-affinity uptake mechanism to a given cell type unless it is known to be devoid of gap junctional contact with other cell types that could contain that mechanism.

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Literature Cited


