

BIOCHEMICAL STUDIES OF ISOLATED GLIAL (MÜLLER) CELLS FROM THE TURTLE RETINA

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ABSTRACT

A method has been developed for the preparation of large numbers of glial (Müller) cells from the turtle retina. After proteolytic dissociation of the retina, Müller cells were separated from retinal neurons by velocity sedimentation at unit gravity. Fractions containing >90% morphologically identifiable Müller cells were prepared by this procedure. Fractions containing only Müller cells were obtained by drawing selected cells individually into a micropipette under visual observation. Biochemical analyses of isolated Müller cells showed that (a) these cells did not synthesize and accumulate acetylcholine, γ -aminobutyric acid, or catecholamines when incubated with appropriate radioactive precursors; (b) the specific activities of choline acetyltransferase (EC 2.3.1.6), glutamate decarboxylase (EC 4.1.1.15), and tyrosine hydroxylase (EC 1.14.16.2) in these cells were less than 2% of those found in the retina; (c) Müller cells, however, contained high activities of transmitter degrading enzymes—acetylcholinesterase (EC 3.1.1.7) and γ -aminobutyrate-transaminase (EC 2.6.1.19); and (d) the cells also possessed high levels of two presumably glial-specific enzymes—glutamine synthetase (EC 6.3.1.2) and carbonic anhydrase (EC 4.2.1.1). These results, together with other findings, suggest that Müller cells are not capable of neurotransmitter syntheses but possess the enzymes necessary for two important roles in the retina: (a) the inactivation of certain transmitters after synaptic transmission by uptake and degradation, and (b) the maintenance of acid-base balance and the provision of a stable microenvironment in the retina by the removal of metabolic products such as carbon dioxide and ammonia.

KEY WORDS vertebrate retina · glial (Müller) cells · neurotransmitter enzymes · glutamine synthetase · carbonic anhydrase

The vast majority of neurons in both vertebrate and invertebrate nervous systems are surrounded by satellite cells known as glia (21). Although neuropathological studies have established that these cells are essential for neuronal survival, the functions of glia and the nature of their interactions with neurons are still poorly understood (21,

45). There is, in particular, very little information about the biochemical properties of glial cells. A direct way to obtain this information is by appropriate biochemical studies of intact, isolated glial cells free from neuronal contamination.

Recently, a method has been developed for the dissociation and separation of the various classes of cells from the vertebrate retina (22, 25). By this method, isolated glial (Müller) cells can be obtained from retinas of several species. These cells, which constitute the bulk of glial cells in the

vertebrate retina, can be identified by their characteristic morphology (24). Inasmuch as isolated Müller cells of the turtle retina are substantially larger than the retinal neurons, fractions containing predominantly Müller cells can be readily prepared by velocity sedimentation at unit gravity (25). Furthermore, a completely homogeneous population of Müller cells can be obtained by selecting individual cells and drawing them into a micropipette under visual observation (22). In the present study, we report the use of this method to examine certain biochemical properties of Müller cells in order to understand some of their functions in the vertebrate retina.

MATERIALS AND METHODS

Cell Dissociation and Separation

The method for the dissociation and separation of retinal cells has been described elsewhere (25). Retinas from fresh water turtles (*Pseudemys scripta elegans*) were incubated for 2 h at room temperature in an isotonic Ca^{++} -free Ringer's solution containing 1 mg/ml of papain and 5 mM ethylene glycol-bis(β -aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA). After incubation, the retinas were transferred to normal Ringer's containing 0.1% bovine serum albumin (BSA) and 10 $\mu\text{g}/\text{ml}$ of deoxyribonuclease, and dissociated by pipetting the tissue gently up and down with a wide-bore pasteur pipette. After the suspension had settled for 5 min, the supernate was removed and the dissociation procedure was repeated with the remaining pieces of undissociated retinas. Approximately four stepwise dissociations were carried out to give four cell fractions. These fractions were examined with differential interference contrast optics of Nomarski, and the fraction most enriched with Müller cells (usually fraction 3 or 4 containing 30–45% Müller cells) was chosen for further purification of Müller cells. This fraction represented about 1% of the wet weight of the retinas used for dissociation.

Velocity sedimentation at unit gravity was carried out in a cold room ($4^\circ \pm 2^\circ\text{C}$) for 4 h using a 30-ml 1–3% BSA or Ficoll gradient as described earlier (25). 1 ml of a cell suspension containing $\sim 10^8$ cells was used for each separation. After the separation, 2-ml fractions were collected from the bottom of the gradient, and the number and types of cells in each fraction were determined with a hemacytometer. Fractions containing >90% Müller cells were used for biochemical studies. Although both BSA and Ficoll gradients yielded good preparations, the morphology of Müller cells from BSA gradients was usually superior.

To obtain a homogeneous population of Müller cells, ~ 0.1 ml of the fraction rich in Müller cells was spread on a glass slide. Individual cells were selected under visual observation (Nomarski optics, magnification 100) and drawn into a micropipette attached to a micromanip-

ulator. Mild suction was applied to draw a cell into the micropipette by connecting the micropipette to a modified aquarium pump with a foot switch. Glass micropipettes were made with a horizontal microelectrode puller, and the tip of each micropipette was broken to give an inner tip diameter of 10–15 μm .

Neurotransmitter Screening

The radiochemical screening procedure of Hildebrand et al. (15) was used to examine the ability of isolated Müller cells to synthesize and accumulate neurotransmitter candidates from exogenous precursors. ~ 100 cells were incubated for 3 h at room temperature (22°C) in 1–2 μl of Leibovitz medium (L-15, Grand Island Biological Co., Grand Island, N. Y.) supplemented with 1 μCi of [*Me*- ^3H]choline chloride (4 Ci/mmol), L-[3,4- ^3H]glutamic acid (45 Ci/mmol), and L-[ring-3,5- ^3H]tyrosine (50 Ci/mmol) (all from New England Nuclear, Boston, Mass.). After incubation, cells were lysed and the radioactivity in the products was determined after their separation by high-voltage paper electrophoresis (pH 1.9, 7,000 V for 1.5 h).

Enzyme Assays

Radioactive substrates (from New England Nuclear) were purified by column chromatography or high-voltage paper electrophoresis before use (25). Glutamic acid used for glutamine synthetase assay was purified by electrophoresis at pH 6.4. Dissociated retinal cell suspensions were centrifuged at 5,000 *g* for 5 min to form pellets. Extracts for enzymatic assays were obtained by homogenizing intact retinas or pellets of isolated cells in a tightly fitted ground homogenizer. The Müller cells in micropipettes were lysed by repeated freezing and thawing and then incubated for 3 h at $22^\circ \pm 2^\circ\text{C}$.

Choline acetyltransferase (EC 2.3.1.6), L-glutamate decarboxylase (EC 4.1.1.15), and tyrosine hydroxylase (EC 1.14.16.2) were determined by methods described earlier (3, 4, 30). γ -aminobutyric acid transaminase (EC 2.6.1.19) was assayed by the method of Hall and Kravitz (9) as modified by Lam (23). Acetylcholinesterase (EC 3.1.1.7) was measured according to the procedure of Schrier et al. (40). The radiometric assay for glutamine synthetase (EC 6.3.1.2) was carried out as described by Krishnaswamy et al. (20). The method of Maren (27) was used to measure the carbonic anhydrase (EC 4.2.1.1) activity. Protein was determined by the method of Lowry et al. (26) using BSA as the standard.

RESULTS

Morphology

The morphology of Müller cells in the vertebrate retina has been described in detail by Cajal (2) using Golgi's silver-impregnation technique. Each Müller cell spans almost the entire thickness of the retina and plays an important role in the

formation of the outer and inner limiting membranes (2). According to Cajal (2), Müller cells of the reptilian retina (Fig. 1A) have a descendant process which arises from the body of the cell at the amacrine cell level and consists of four-eight branches with short varicose collaterals in the

inner plexiform layer. Most of this characteristic morphology can be seen in an apparently intact isolated Müller cell of the turtle retina (Fig. 1B), thus making it possible to identify these cells readily and unequivocally. In addition, the nucleus, not usually revealed by Golgi's technique,

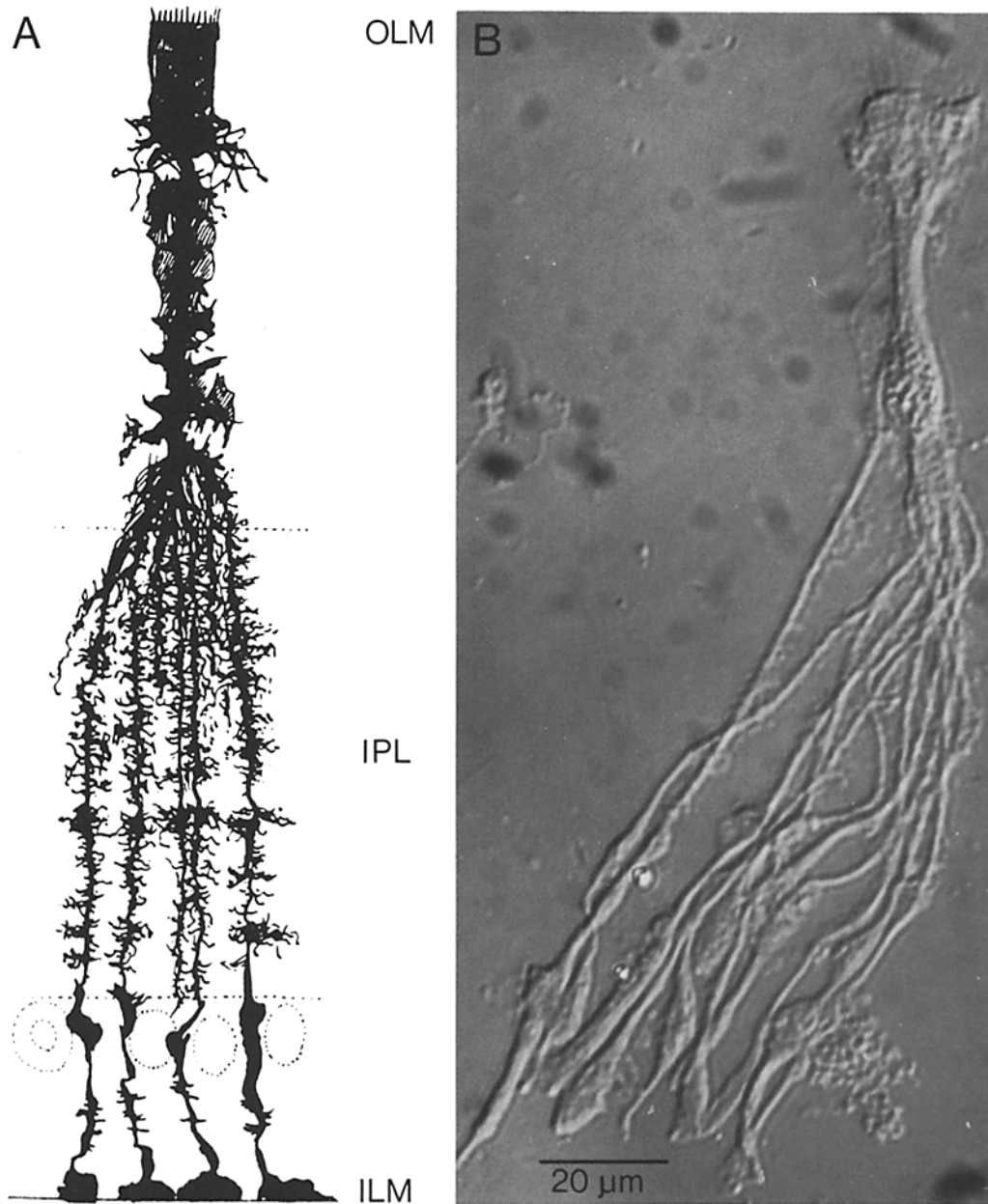


FIGURE 1 (A) A Golgi-impregnated Müller cell from a reptilian retina (2). (B) An isolated Müller cell from the turtle retina. OLM, outer limiting membrane; IPL, inner plexiform layer; ILM, inner limiting membrane. Bar, 20 µm.

is clearly seen in isolated Müller cells. Finally, dye exclusion tests using eosin (38) and trypan blue (35) showed that >95% of the isolated Müller cells excluded these dyes and therefore could be considered viable by this criterion.

Cell Separation

After dissociation of the retina, the cell fraction most enriched with Müller cells (fraction 3 or 4, 30–45% of the total number of cells) was used for further purification by velocity sedimentation at unit gravity. In addition to Müller cells, this fraction contained photoreceptors, bipolar, amacrine, and ganglion cells. A few intact horizontal cells (< 5% in number), which do not separate completely from Müller cells during velocity sedimentation, were also found in this fraction. A typical profile of such a cell suspension after velocity sedimentation is shown in Fig. 2. Müller cells sediment at a velocity of ~38 mm/h which is slightly faster than that for horizontal cells (~33 mm/h) and approximately twice the rate for photoreceptors (~17 mm/h). Bipolar, amacrine, and ganglion cells in the turtle retina are much smaller and sediment at velocities <12 mm/h. Thus, preparations containing >90% Müller cells as judged by cell count can be obtained for biochemical studies by pooling fractions 5 and 6 of Fig. 2. As shown in Fig. 3A, fraction 6 is comprised almost exclusively of morphologically identifiable Müller cells. To obtain a homogeneous population of intact Müller cells, Müller cells are selected under visual observation and drawn into a micropipette individually (Fig. 3B).

Enzymes in Retina and Dissociated Cells

The use of proteolytic enzymes and mechanical dissociation to obtain isolated retinal cells may lead to leakage of intracellular constituents and/or loss of enzymatic activities. Thus, before measuring the activities of various enzymes in isolated Müller cells, it is essential to compare the specific activities of these enzymes in homogenates of intact retinas and dissociated retinal cells (containing all cell types) before and after velocity sedimentation. As shown in Table I, the specific activities of all seven enzymes measured in the dissociated cells and in cells subjected to sedimentation are at least 80% of those found in homogenates of retina. These results show that our cell dissociation and separation procedure does not decrease significantly the activities of these enzymes.

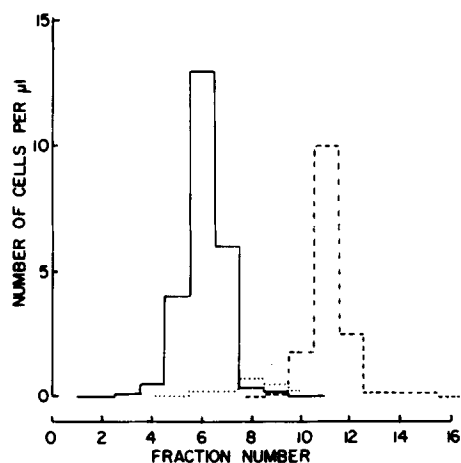


FIGURE 2 Separation of dissociated cells from the turtle retina by velocity sedimentation. The histograms represent the distributions of Müller cells (solid line), horizontal cells (dotted line), and photoreceptors (broken line).

Synthesis of Neurotransmitters

The ability to synthesize, store, and release neurotransmitters is generally believed to be an exclusive property of neurons (45). However, under certain conditions, glial cells might exhibit some of these properties (15, 17, 29, 39, 41). Radiochemical screening has shown that of the various neurotransmitter candidates, turtle retinas synthesize and accumulate only acetylcholine (ACh), γ -aminobutyric acid (GABA), and dopamine (22). We therefore examined the ability of isolated Müller cells to synthesize these transmitters. As with the retina, dissociated retinal cells containing all cell types were found to synthesize ACh, GABA, and dopamine when incubated with 100 μ Ci/ml of 3 H-labeled choline, glutamate, and tyrosine (Table II). Müller-cell-enriched fractions were also found to synthesize ACh and GABA, but not dopamine. Because the Müller cell preparations used in these studies may contain up to 10% retinal cell types, especially horizontal cells, it was necessary to study transmitter synthesis in a homogeneous population of Müller cells. ~100 isolated Müller cells were therefore drawn into a micropipette and incubated with 1 μ Ci/ μ l of 3 H-labeled precursors for 3 h (Fig. 3). The radioactive products were analyzed by high-voltage paper electrophoresis. As shown in Table II, the radioactivity associated with 3 H-labeled ACh, GABA, or dopamine was not significantly above the background levels, indicating that isolated

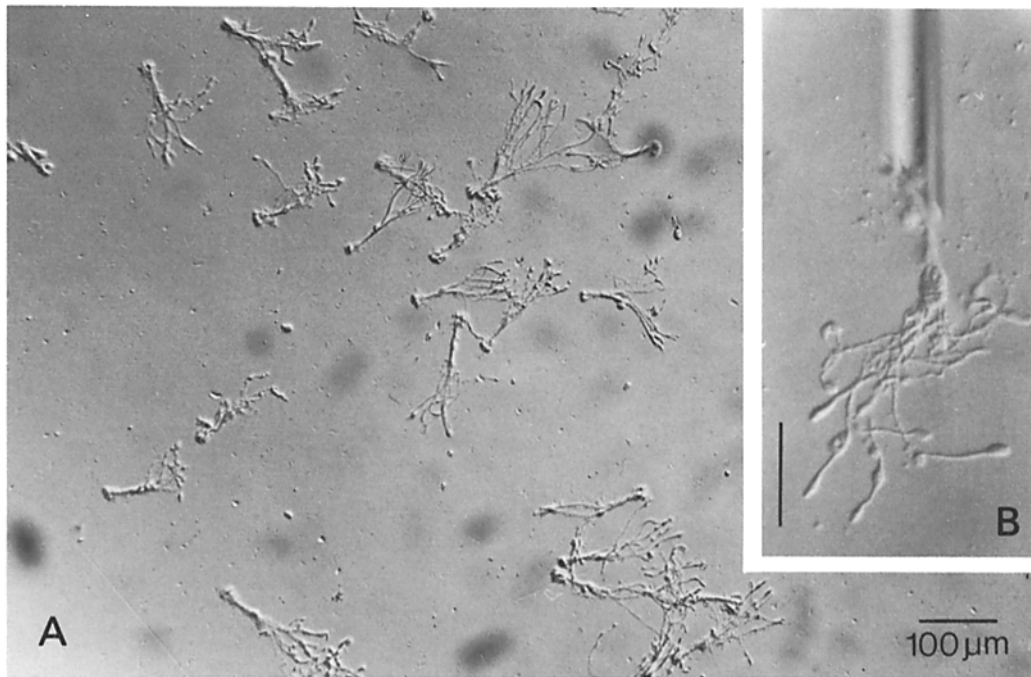


FIGURE 3 (A) Low-power view of a Müller-cell-enriched fraction from the BSA gradient. Bar, 100 μm . (B) A Müller cell being drawn into a micropipette under visual observation. Bar, 50 μm .

TABLE I
Specific Activities of Certain Enzymes in Müller-Cell- and Photoreceptor-Enriched Fractions

	Müller cells	Photoreceptors	Dissociated retinal cells from gradient	Dissociation retinal cells	Retina
Choline acetyltransferase*	1.2 \pm 0.1	4.0 \pm 0.7‡	18.3 \pm 2.6	20.1 \pm 1.1	15.9 \pm 2.4
Glutamic acid decarboxylase*	1.0 \pm 0.3	<1.0‡	14.1 \pm 1.9	15.3 \pm 0.7	16.3 \pm 1.4
Tyrosine hydroxylase*	<0.01	<0.05‡	0.2 \pm 0.1	0.2 \pm 0.1	0.2 \pm 0.1
GABA-transaminase*	11.4 \pm 1.5	0.9 \pm 0.2	35.5 \pm 6.1	40.2 \pm 7.2	42.5 \pm 5.6
Acetylcholinesterase*	6045 \pm 404	2773 \pm 209	7022 \pm 795	7992 \pm 653	8549 \pm 641
Glutamine synthetase*	283 \pm 11	79 \pm 9	102 \pm 30	125 \pm 16	114 \pm 24
Carbonic anhydrase§	8036 \pm 561	320 \pm 61	1324 \pm 107	1411 \pm 79	1563 \pm 112

Each value is a mean \pm SD for four-five experiments.

* Micromoles of product formed per hour per gram of protein.

‡ Enzyme activity in isolated cells.

§ Enzyme units per gram of protein.

TABLE II
Synthesis and Accumulation of Neurotransmitter Candidates by Isolated Müller Cells

Incubations (3 h)	Neurotransmitters synthesized		
	[³ H]acetylcholine	[³ H] γ -aminobutyric acid	[³ H]dopamine
Isolated Müller cells (~100)	169 \pm 12	65 \pm 17	112 \pm 26
Incubation medium	148 \pm 16	87 \pm 6	108 \pm 4
Müller-cell-enriched fraction	679 \pm 36	291 \pm 52	115 \pm 15
Dissociated retinal cells	6874 \pm 1121	13296 \pm 1188	2428 \pm 557

All values are means \pm SD of counts per minute for four-five experiments.

Müller cells do not synthesize detectable amounts of ACh, GABA, or dopamine.

Enzymes for Transmitter Synthesis

The lack of neurotransmitter syntheses by isolated Müller cells might be due to the absence of the enzymes for transmitter syntheses, or to other factors. We therefore measured the activities of choline acetyltransferase, glutamate decarboxylase, and tyrosine hydroxylase in Müller-cell-enriched fractions as well as in identified Müller cells. For comparison, specific activities of these enzymes were also measured in extracts of dissociated retinal cells and in isolated photoreceptors (25). The Müller-cell-enriched fractions were found to contain low activities of choline acetyltransferase and glutamate decarboxylase compared with the retina (Table I). Extracts of isolated Müller cells, however, contained very little,

if any, such activities (Table III). These results show that isolated Müller cells do not synthesize acetylcholine, γ -aminobutyrate, or dopamine and also do not contain significant quantities of the corresponding synthetic enzymes.

Enzymes for Transmitter Degradation

Unlike enzymes that catalyze transmitter synthesis, enzymes that degrade transmitters are present in neurons as well as in glia (10, 12, 17, 23, 43), where they may be involved in the catabolism of neurotransmitter molecules taken up from the synaptic cleft (13, 17). To examine this function, we determined the specific activities of acetylcholinesterase and GABA-transaminase in Müller cells. The experimental data presented in Tables I and III show that both Müller-cell-enriched fractions and isolated Müller cells contain significant quantities of these enzymes, although

TABLE III
Enzymatic Activities in Isolated Müller Cells

	Isolated Müller cells	Isolated retinal cells
Choline acetyltransferase	232 ± 68	14404 ± 2040
Blank	143 ± 36	301 ± 102
Specific activity	0.31 ± 0.10	18.3 ± 2.6
Glutamate decarboxylase	135 ± 29	4320 ± 580
+ AOAA (100 μ M)	125 ± 25	356 ± 52
+ EAOS (5 mM)	139 ± 26	5132 ± 548
Blank	117 ± 37	123 ± 12
Specific activity	<0.16	14.1 ± 1.9
GABA-transaminase	2523 ± 312	17040 ± 2900
+ EAOS (5 mM)	263 ± 17	1090 ± 109
Blank	133 ± 36	131 ± 38
Specific activity	14.6 ± 1.8	35.5 ± 6.1
Acetylcholinesterase	3644 ± 355	14746 ± 1658
+ physostigmine (50 μ M)	806 ± 196	3670 ± 371
+ BW284C51 (20 μ M)	583 ± 112	1375 ± 51
Blank	206 ± 48	239 ± 62
Specific activity	4820 ± 470	7022 ± 795
Glutamine synthetase	3780 ± 518	6120 ± 1784
Blank	121 ± 34	182 ± 44
Specific activity	175 ± 24	102 ± 30

All values for Müller cells are means ± SD of counts per minute per 100 cells per 3 hours for three-four experiments. For retinal cells, the values are means ± SD of counts per minute per microgram of protein per hour for three-four experiments. The specific activities are in micromoles of product per hour per gram of protein. Protein content of isolated cells was calculated by assuming that a Müller cell is a cylinder of radius 5 μ m and height 150 μ m. This gives a wet weight of 1.2×10^{-8} g/cell (density ~1), and if 10% of the wet weight is protein, the protein content of 100 isolated cells is ~0.12 μ g. AOAA, aminoxy-acetic acid; EAOS, ethanolamine-O-sulfate; BW284C51: 1,5-bis(4-allyldimethyl ammonium phenyl)pentan-3-one dibromide.

the activities in the isolated cells were somewhat lower than those in the enriched fractions. In comparison, activities of the enzymes in the photoreceptor-rich fraction, prepared according to Lam (22), were severalfold lower (Table I).

The activity of GABA-transaminase was more than 90% inhibited by 20 mM ethanolamine-O-sulfate (gift of Dr. Fowler), a specific inhibitor of mammalian GABA-transaminase (7). The acetylcholine hydrolyzing activity was blocked 70–80% by 50 μ M physostigmine and ~80% by 20 μ M 1,5-bis(4-allyldimethyl ammonium phenyl)pentan-3-one dibromide (BW284C51, Sigma Chemical Co.) (Table III).

Glutamine Synthetase and Carbonic Anhydrase

Glutamine synthetase catalyzes the amidation of glutamate to glutamine in the presence of ATP and ammonia. Carbonic anhydrase catalyzes the hydration of CO₂ to HCO₃⁻. These enzymes have been reported to be localized specifically to glial cells by histochemical methods (8, 14, 31, 36). We therefore measured the specific activities of glutamine synthetase and carbonic anhydrase in homogenates of enriched fractions of Müller cells and photoreceptors, and in dissociated retinal cells, to determine whether these enzymes were present exclusively in glial cells of the turtle retina. As shown in Table I, the specific activity of glutamine synthetase in isolated Müller cells was about threefold higher than in the dissociated retinal cells and fourfold higher than in the photoreceptors. The specific activity of carbonic anhydrase in Müller cells was about fivefold higher than in dissociated retinal cells and at least 25-fold higher than in isolated photoreceptors.

DISCUSSION

Several techniques such as hand dissection, bulk cell fractionation, and tissue culture are available for the isolation of glial cells (21, 45). In the present study, we have described a method by which glial (Müller) cells can be isolated by proteolytic treatment of adult retinas of the turtle. These cells appear to retain their characteristic morphology at the light microscope level, and are viable by dye exclusion tests. Large numbers of isolated Müller cells can be separated from retinal neurons by velocity sedimentation, yielding fractions containing >90% Müller cells. In addition, small but homogeneous populations of intact

Müller cells can be obtained by selecting Müller cells individually and drawing them into a micropipette. We have used this procedure to begin a biochemical characterization of isolated Müller cells. In this paper, we have reported specific activities of (a) several presumably neuronal specific enzymes responsible for neurotransmitter synthesis, (b) the enzymes responsible for ACh and GABA degradation and (c) two presumably glial-specific enzymes.

Our experimental results clearly show that in the turtle retina the cell dissociation and separation procedure does not decrease significantly the specific activities of any of the enzymes examined in this study. In addition, we (unpublished data) have observed that the concentrations of free amino acids and ACh in dissociated retinal cells are comparable to those found in the intact retina. Kaneko et al. (18) have shown that horizontal cells isolated by this procedure still possess a high membrane resistance. These findings indicate that there is no significant loss of intracellular constituents from isolated retinal cells and that our biochemical measurements of isolated cells may reflect closely those found in the intact retina.

Although Müller-cell-enriched fractions synthesize ACh and GABA from precursors and also possess low levels of choline acetyltransferase and glutamate decarboxylase, identified Müller cells clearly do not synthesize ACh or GABA and contain no demonstrable activities of the enzymes for ACh and GABA syntheses. Assuming that the activities of these enzymes in Müller-cell-enriched fractions arise exclusively from neuronal contamination, our results indicate that these fractions contain ~93% "biochemically pure" Müller cells. This estimate is comparable with the morphological observation that Müller-cell-enriched fractions contain up to 10% contamination from horizontal and unidentifiable cells.

It is generally accepted that a basic difference between glia and neurons is the ability of the latter to synthesize, store, and release neurotransmitter molecules (45). However, under certain conditions, glial cells have been observed to exhibit some of these properties. For example, Schwann cells at the neuromuscular junction can synthesize and release quanta of acetylcholine after nerve ending degeneration, although it is not known whether they do so normally (1, 5). In mammalian brain, glial cells preloaded with GABA can release the amino acid in response to depolarization with K⁺ (17, 29). Some rat glial tumor cell

lines have been reported to synthesize, take up, and release GABA and taurine (39). Many clonal lines of presumptive glial origin have been reported to possess neurotransmitter synthetic enzymes (41). The experimental data presented in this paper establish that isolated glial cells from the turtle retina are unable to synthesize transmitters. To our knowledge, this is the first clear demonstration of the absence of ACh, GABA, or catecholamine synthesis by a homogeneous population of glial cells from an adult central nervous tissue.

Glial cells have been postulated to be involved in the inactivation of synaptically released neurotransmitters by uptake and degradation (13, 17). In invertebrates, autoradiographic studies have shown that the sheath (glial) cells surrounding the neuromuscular junction selectively accumulate GABA (34) and glutamate (6), suggesting that glia may be the principal pathway for removal of neurotransmitters. However, in vertebrate nervous systems, uptake and release of GABA have been observed in both glia and neurons (17, 28, 32). In particular, Müller cells of certain vertebrate retinas have been shown to take up exogenous GABA (24, 28, 32). GABA-transaminase, the first enzyme in the degradative pathway for GABA has been found in both neurons and non-neural tissues (9, 37). In the rat retina, this enzyme has been localized to the Müller cell cytoplasm by histochemical methods (16). These findings, together with our result that isolated Müller cells from the turtle retina contain high levels of GABA-transaminase activity, suggest that Müller cells in the vertebrate retina may participate in the inactivation of GABA after its synaptic release.

Several enzymes such as acetylcholinesterase, cholinesterase, and acylesterase can catalyze the hydrolysis of ACh (42). However, these enzymes differ from one another in their substrate specificity and sensitivity to inhibitors (42). Using selective inhibitors, we have examined the nature of ACh hydrolyzing activity in the Müller cell extracts. Physostigmine, an inhibitor of acetylcholinesterase and cholinesterase activities, inhibited 70–80% of the activity at a concentration of 50 μ M. ACh hydrolysis was blocked 80% or more by 20 μ M 1,5-bis(4-allyl dimethyl ammonium phenyl)pentan-3-one dibromide, which preferentially inhibits acetylcholinesterase without affecting the cholinesterase activity to a significant extent. These results suggest that much of the

ACh hydrolyzing activity in the isolated Müller cells is of the acetylcholinesterase type. Although acetylcholinesterase is generally found in neurons (42), it has also been observed in non-neuronal cells such as erythrocytes and in tissues such as mammalian cerebellum which appears to be non-cholinergic (42). The function of acetylcholinesterase in these cases is not known. Hemminki et al. (12) examined the activity of dopamine decarboxylase, monoamine oxidase, choline acetyltransferase, cholinesterase, and acetylcholinesterase in various cell fractions from rat brain cortex. They were unable to localize any of the above enzymes to particular cell fractions, suggesting that both glial and neuronal cell preparations have dopamine decarboxylase, monoamine oxidase, and acetylcholinesterase activities. This is consistent with our observation that both photoreceptor and Müller cell preparations contain significant levels of acetylcholinesterase activity.

Glutamate metabolism appears to be compartmentalized in the brain into a large compartment involved in energy metabolism and a small compartment concerned with GABA metabolism, glutamine synthesis, and ammonia detoxification (44). Using light microscope immunohistochemical techniques, Hernandez et al. (14) reported that glutamine synthetase was localized exclusively in glial cells of the rat brain but not in neuronal cell bodies, endothelial cells, or in the choroid epithelium. Recently, glutamine synthetase has been localized to Müller cells in the rat retina by immunohistochemical techniques (36). No staining was seen in photoreceptors, horizontal cells, bipolar cells, amacrine cells, or ganglion cells. From these results, it has been suggested that the small glutamate compartment associated with glutamine synthesis resides solely in the glial cells of brain (44) and retina (19). Our biochemical data are in agreement with the localization of glutamine synthetase to Müller cell cytoplasm, although we did observe some activity of the enzyme in photoreceptor-enriched fractions. The reason for this discrepancy is not known.

The exact role of retinal carbonic anhydrase is unknown, but the enzyme has been implicated in at least two functions in the retina: removal of metabolic CO_2 by hydration of HCO_3^- and the regulation of acid-base balance. Inasmuch as the retina has a high rate of respiration (33), large amounts of CO_2 may have to be removed from the tissue. The high level of carbonic anhydrase activity in the Müller cells may serve this function.

Our finding that carbonic anhydrase is found mostly in Müller cells is in agreement with the histochemical localization of this enzyme in the Müller cell cytoplasm (31). Carbonic anhydrase has been suggested as a "marker" for glial cells from the brain (8), but the enzyme has not proved to be a useful marker in most cell separation methods (11). It has, in fact, been suggested to represent capillary contamination (11). Our observations and the results of Musser and Rosen (31) indicate that, at least in the retina, carbonic anhydrase may be considered a biochemical marker for glia.

Glial cells have been implicated in various functions such as the provision of structural framework, insulation, and nutrients to neurons, the removal of waste products from neuronal metabolism, the uptake and degradation of transmitters after synaptic activity, and the repair, regeneration, and development of neurons (21, 45). Our results are compatible with at least two roles for glia in the vertebrate retina: (a) uptake and degradation of certain neurotransmitters after synaptic transmission, and (b) removal of metabolic products such as CO₂ or NH₃, thus maintaining a stable microenvironment for the retina. Although a complete understanding of glial cell function must await more extensive biochemical, physiological, and morphological studies, the work presented here points to the possibility that isolated Müller cells may provide a useful system for some of these studies.

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