Chapter 6
Glia of the Retina
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There are three principal types of glial cells in the mammalian retina: Müller cells, astrocytes, and microglia. Müller cells and astrocytes are classified as macroglial cells and are developmentally, physiologically, and functionally distinct from microglia. This chapter discusses the morphologic and physiologic properties of these cells, as well as their contributions to retinal function. The reader is referred to recent reviews for additional information.46,68,69,72

Müller cells are the most prominent glial cell of the retina. They are a specialized form of radial glia that span nearly the entire depth of the retina. Astrocytes, the second type of retinal macroglial cell, are present only in species having a retinal circulation and, in these species, are restricted largely to the nerve fiber layer at the inner boundary of the retina. Microglia, the third type of retinal glial cell, are present in the nerve fiber layer (NFL) and the inner and outer plexiform layers of the retina.

Oligodendrocytes, the glial cells that form the insulating sheath of myelinated axons in the brain, are completely absent from the retina, except in a few species, including rabbit, which have myelinated axons in the NFL. They are not discussed in this chapter.

Glia1 cells, in the retina and elsewhere in the CNS, have traditionally been thought to serve only passive structural and metabolic functions. However, it has become evident that glial cells play an active and essential role in CNS function. As discussed below, retinal glial cells are thought to participate actively in many processes, including the regulation of extracellular K⁺, H⁺, and neurotransmitter levels, the induction of retinal vascularization, and the active metabolic support of retinal neurons.

DEVELOPMENT AND MORPHOLOGY
Müller Cells

Müller cells are a specialized type of radial glial cell found only in the retina (Fig. 6-1). They differentiate from progenitor cells at the apical margin of the retinal neuroepithelium during the second phase of cell differentiation, following the birth of ganglion cells, horizontal cells and cones.17,117 The developing Müller cells migrate proximally and extend processes toward the inner and outer retinal margins. Their radial processes serve to guide migrating neurons during subsequent retinal development.

Müller cell somata lie in the middle region of the inner nuclear layer. A radial process extends toward the inner border of the retina, where a basal endfoot forms the inner limiting membrane (a basal lamina). A second cell process extends to the outer limiting membrane, where apical microvilli project into the subretinal space, substantially increasing the surface area of the cell. Secondary Müller cell processes project from the main trunk of the cell, covering the membranes of neurons in all retinal layers (Fig. 6-2). In the NFL, Müller cell lamella wrap around bundles of ganglion cell axons50,78 and contact specialized nodelike regions of the axons.6 In the nuclear layers, cell processes encase neuronal somata, and in the inner plexiform layer, numerous fine processes envelope neuronal membranes. Basal Müller cell endfeet terminate onto both the inner limiting membrane and blood vessels in the superficial retina. Blood vessels within the inner nuclear layer are surrounded by additional Müller cell processes that may function as endfeet.93 (See references 54, 91, 92, 96, and 119 for descriptions of Müller cell morphology.)

Müller cells contain numerous glycogen granules that represent the main retinal glycogen stores in some species.18,48
They also contain numerous mitochondria and intermediate filaments, composed both of vimentin and glial fibrillary acidic protein (GFAP). GFAP distribution is sparse in Müller cells of normal retinas but can be dramatically upregulated following retinal injury. Orthogonal arrays of particles (OAPs) are distributed across the plasma membrane of Müller cells and are present at high density on endfoot processes, both at the retinal surface and at retinal vessels. The function of these particles is not known, although they may represent aquaporin water channels.

**Astrocytes**

Astrocytes do not arise from the retinal neuroepithelium but rather immigrate to the retina. They migrate into the developing retina from the optic nerve and advance across the retinal surface as the retina matures. The spacing of astrocytes over the surface of the retina is determined by a "contact-spacing" interaction between cells. The distal processes of astrocytes remain in contact with each other, but astrocyte somata are spaced as far apart as possible.

In the mature retina, astrocytes are restricted largely to the NFL. They are closely associated with blood vessels and are present only in species having a retinal circulation. Their processes are confined largely to the plane parallel to the retinal surface, although processes sometimes follow blood vessels into the ganglion cell layer. In many species astrocytes are largely stellate in appearance. In others, including cat and primates, astrocytes of the central retina are bipolar, their processes running parallel to ganglion cell axons. Astrocyte endfeet terminate on superficial retinal blood vessels. Astrocytes contain glycogen granules and dense bundles of intermediate filaments composed of GFAP.

**Microglia**

Microglia are the resident macrophages of the retina. They are thought to have a mesodermal origin, deriving from blood monocytes and entering the retina along with blood vessels during development. Additional microglial cells may enter the mature retina as blood-borne cells. Microglia are normally present in the retina in their dormant state and are found principally in the NFL and the inner and outer plexiform layers. They have small somata and short, irregular processes. The cells normally are flat and have slender, hairlike extensions protruding from their processes. Microglial cells proliferate after retinal injury and differentiate into macrophage-like cells that remove degenerating retinal neurons by phagocytosis. Microglia also phagocytose neurons that die during the course of normal retinal development.
Fig. 6-2  Drawing of a Müller cell of a vascularized mammalian retina. Neuronal somata and processes are ensheathed by the processes of the Müller cell (shaded blue). M, Müller cell; EF, Müller cell endfoot; MV, Müller cell microvilli; A, amacrine cell; B, bipolar cell; C, cone photoreceptor cell; G, ganglion cell; H, horizontal cell; R, rod photoreceptor cell; CAP, capillary. (Drawing by Andreas Reichenbach. From Newman, EA, and Reichenbach, A: TINS 19:307-312, 1996.)

Fig. 6-3  Fluorescence micrograph of astrocytes within the nerve fiber layer of the cat retina. The tissue was labeled with fluorescently tagged antibodies against glial fibrillary acidic protein (GFAP). The image is from a peripheral portion of a retinal whole mount. (From Karschin, A, Wassil, H, Schnitzer, J: Invest Ophthalmol Vis Sci 27:828-831, 1986.)
Fig. 6-4  Drawings of astrocytes in different regions of the cat retina. Cells are labeled with antibodies against glial fibrillary acidic protein (GFAP). A, In the peripheral retina, astrocytes are star-shaped, with a few processes oriented toward a blood vessel (cross hatching). B, In the mid-retina, astrocyte processes are oriented along axons in the nerve fiber layer. C, Close to the optic disc, where there is a high density of nerve fibers, there is a corresponding high density of astrocytic processes, which are aligned with the nerve fibers. (From Karschin, A, Wassle, H, and Schnitzer, J: Invest Ophthalmol Vis Sci 27:828-831, 1986.)

Fig. 6-5  Micrograph of microglial cells within the inner plexiform layer of the rabbit retina. The cells are labeled to reveal nucleoside diphosphatase (NDPase) activity. Cell morphology is typical of resting microglial cells. Scale bar, 50 µm. (From Schnitzer, J: J Comp Neurol 282:249-263, 1989.)
Coupling Between Glial Cells

In the mammalian retina astrocytes are coupled to each other and to Müller cells by gap junctional complexes within the nerve fiber and ganglion cell layers. When a tracer molecule is injected into a single astrocyte, it spreads into many neighboring astrocytes and Müller cells (Fig. 6-6). Interestingly, the coupling is asymmetric; when tracer is injected into a Müller cell, it does not spread to either astrocytes or to other Müller cells. In rabbit, astrocytes are coupled to oligodendrocytes as well as to Müller cells.

In amphibians, Müller cells are the sole macrogial cells and are coupled to each other by gap junctions at the outer limiting membrane. Coupling is relatively weak. The spread of tracer and the presence of electrical coupling are detected only in nearby cells.

MÜLLER CELL PHYSIOLOGY

For many years, Müller cells, like other glial cells, were thought to function only as inactive elements within the retina. As detailed below, Müller cells are now known to possess voltage-gated channels, neurotransmitter and growth factor receptors, and acid-base transporters. They also release several essential trophic factors. It is evident that these cells interact actively with retinal neurons and serve many essential functions.

Ion Channels

Potassium Channels. The plasma membrane of Müller cells is highly permeable to $K^+$. This results in a cell membrane potential that is extremely negative and close to the $K^+$ equilibrium potential. The high $K^+$ conductance is primarily due to the presence of inward rectifying $K^+$ channels, a voltage-gated channel that is open at the resting membrane potential of the cell and is activated by ATP. The channel has been identified as a $K_{IR4.1}$, ATP-binding $K^+$ channel.

In species with avascular retinas, including rabbit, frog, and salamander, the $K^+$ channels are localized predominantly to the cell endfoot. In vascularized species, including primates, a high density of $K^+$ channels is present on the basal endfoot and on processes near the soma, lying within the inner nuclear layer in situ (Fig. 6-7). The $K^+$ channels of Müller cells are modulated by a number of substances. Both glutamate and dopamine decrease cell $K^+$ conductance, as does thrombin. Müller cells regulate extracellular $K^+$ levels ($[K^+]_o$) within the retina by acting as conduits for $K^+$-siphoning currents, which are conducted by the inward rectifying channels (see below). Modulation of the channels by neurotransmitters and thrombin may function to control Müller cell regulation of $[K^+]_o$.

Other Channels. Several other voltage-gated ion channels are also present in Müller cells. $Ca^{2+}$-activated $K^+$ channels, fast inactivating $K^+$ channels, and high voltage-activated $Ca^{2+}$ channels are present in amphibian Müller cells. In addition, L-type $Ca^{2+}$ channels and $Na^+$ channels are present in mammalian cells.

The function of these channels is unclear, since they are activated at voltages that are normally not attained by Müller cells. Cells rarely depolarize more than 10 mV from a resting potential.
potential of near $-85$ mV. Most voltage-gated channels, on the other hand, are not activated until cells depolarize to near $-50$ or $-40$ mV. Active generation of Ca$^{2+}$ action potentials, produced by voltage-gated Ca$^{2+}$ channels, is observed in Müller cells when the K$^+$ conductance of a cell is blocked. 60 It is not known whether such conditions occur in vivo, however.

**Receptors**

**Neurotransmitter Receptors.** Several glutamate receptors are present in mammalian Müller cells, including AMPA (GluR4), 82 NMDA, 88 and metabotropic receptor types. 42,106 Also present are GABA$_A$, 53,94 dopaminergic, 3,106 cholinergic, and adrenergic 81 receptors, as well as receptors for several neuroactive peptides. 42 In general, these receptors display high binding affinities and pharmacologic properties similar to those described in neurons.

Stimulation of these receptors can lead to a variety of effects. Activation of the GABA$_A$ receptor results in the opening of an anion channel and to cell depolarization. 53,94 Activation of a metabotropic glutamate receptor and a dopamine receptor leads to a decrease in cell K$^+$ conductance. 106 Activation of the AMPA 120 and metabotropic 42 receptors result in an increase in intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]) in Müller cells.

**Other Receptors.** Müller cells also possess receptors for a number of growth factors, including basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and nerve growth factor (NGF). 15,84,99,118 The effects of these factors are varied and include the stimulation of DNA synthesis, mitosis, and cell proliferation, expression of cytoskeletal filaments, and the modulation of ion channels. 84 Müller cells also possess a thrombin receptor, which stimulates an increase in [Ca$^{2+}$] and a decrease in K$^+$ conductance. 87

**Transmitter Transporters**

**Glutamate Transporter.** A high-affinity glutamate/aspartate transporter (GLAST) is present in Müller cells 49 and serves to maintain a low concentration (<1 μM) of glutamate in extracellular space. 10 Once glutamate is transported into Müller
cells, it is rapidly converted to glutamine and ammonia by glutamine synthetase, which is present at high levels.\textsuperscript{52,97,106}

The transporter has a complex stoichiometry, with inward transport of a glutamate molecule and 2 Na\(^{+}\) coupled to the outward transport of 1 K\(^{+}\) and 1 OH\(^{-}\).\textsuperscript{9,10} Because of this stoichiometry, transport of glutamate is accompanied by cell depolarization and an extracellular alkalization. The transporter is localized preferentially to the distal half of Müller cells.\textsuperscript{12}

**GABA Transporter.** A high-affinity GABA transporter of the GAT-3 type is also present in Müller cells.\textsuperscript{31,100} The transporter has a stoichiometry of 1 GABA molecule, 2 Na\(^{+}\), and 1 Cl\(^{-}\), all transported inwards.\textsuperscript{1} Once transported into the cell, GABA is converted to succinic semialdehyde by the enzyme GABA-transaminase.\textsuperscript{101}

**Acid-Base Transport**

Several acid-base transport systems are present in Müller cells. The transporters regulate intracellular pH (pH\(_{i}\)) and can influence extracellular pH (pH\(_{e}\)). A Na\(^{+}\)/HCO\(_{3}\) cotransporter, having a stoichiometry of 2 or 3 HCO\(_{3}\) transported along with 1 Na\(^{+}\), is present in salamander, where it is preferentially localized to the basal endfoot of the cell.\textsuperscript{62,66} The transporter is electrogenic. Cell depolarization, generated, for example, by an increase in [K\(^{+}\)]\(_{o}\), leads to an influx of HCO\(_{3}\) and Na\(^{+}\) via the transporter.\textsuperscript{66} The HCO\(_{3}\) influx, in turn, generates an intracellular alkalization and an extracellular acidification, which is largest at the endfoot (Fig. 6-8).

A Na\(^{+}/H^{+}\) exchanger and a Cl\(^{-}/HCO_{3}\) anion exchanger are also present in Müller cells.\textsuperscript{46,67} The anion exchanger is of the AE3 type and is preferentially localized to the cell endfoot.\textsuperscript{45} Intracellular and membrane-bound forms of the enzyme carbonic anhydrase (CA) are also present in Müller cells at high activity levels.\textsuperscript{52,64,66} The enzyme catalyzes the conversion of HCO\(_{3}\) and H\(^{+}\) to CO\(_{2}\) and H\(_{2}\)O and may play a role in pH and CO\(_{2}\) regulation in the retina (see below).

**Calcium Waves**

Both intracellular and intercellular Ca\(^{2+}\) waves can be propagated through Müller cells. In freshly isolated and cultured cells, stimuli, including glutamate, ATP, high [K\(^{+}\)]\(_{o}\), and caffeine, trigger increases in intracellular Ca\(^{2+}\) concentration.\textsuperscript{41,42,120} In isolated salamander cells, these [Ca\(^{2+}\)]\(_{i}\) increases begin in the apical portion of the cell and propagate to the basal endfoot\textsuperscript{41,42} (Fig. 6-9, A). In situ, in the rat retina, Ca\(^{2+}\) waves triggered by electrical, mechanical, or chemical stimuli are propagated through both astrocytes.
and Müller cells\(^7\) (Fig. 6-9, B). The waves travel across the retinal surface within the astrocytes in the NFL and invade the basal portion of Müller cells, leading to Ca\(^{2+}\) increases within the Müller cell endfoot and basal process.

### Growth Factors

Müller cells secrete a number of growth factors that have important influences on neurons and other cells in the retina. bFGF, which promotes photoreceptor survival,\(^{108}\) is synthesized by Müller cells and retinal astrocytes.\(^{15,50} \) Both Müller cells and astrocytes synthesize vascular endothelial growth factor (VEGF),\(^{112} \) which plays an essential role in the development of the retinal vasculature (see above).

### ASTROCYTE PHYSIOLOGY

Relatively little research has been conducted on the physiology of retinal astrocytes. The work that has been done, however, indicates that their properties are similar to those of astrocytes in other parts of the CNS. See Kettenmann and Ransom\(^3\) for a comprehensive review of the properties of CNS astrocytes.

### Ion Channels

The resting membrane potential of retinal astrocytes is only slightly less negative than that of Müller cells (−85 vs. −93 mV for rat astrocytes and Müller cells, respectively\(^{125} \), indicating that retinal astrocytes, like Müller cells, have a high K\(^+\) membrane conductance. The distribution of K\(^+\) channels in retinal astrocytes is not known. Voltage-gated Na\(^+\) channels, as well as fast-inactivating and delayed rectifier K\(^+\) channels, are present in astrocytes of the rabbit retina.\(^{20} \)

### Receptors

Retinal astrocytes possess several types of receptors. Rabbit cells display AMPA-type glutamate receptors and GABA re-
ceptors that generate cell depolarization when stimulated. ATP, acetylcholine, and epinephrine receptors are present in rat astrocytes and mediate increases in [Ca^{2+}]. Astrocytes also express receptors for platelet-derived growth factor (PDGF) that mediate the proliferation of astrocytes within the NFL during development.

**Calcium Waves**

Stimulation of an astrocyte at the retinal surface triggers an increase in [Ca^{2+}], within that cell that propagates outward through a network of astrocytes and Müller cells (Fig. 6-9, B). The propagated Ca^{2+} waves travel at a velocity of approximately 23 μm/sec and can extend outward for distances as great as 180 μm. The [Ca^{2+}], increases arise from a release of Ca^{2+} from internal stores, mediated, at least in part, by IP₃ receptors.

**MICROGLIA**

Little work has been done on the physiologic properties of retinal microglial cells. A comprehensive review of the properties of microglia in other CNS regions can be found in Kettenmann and Ransom.

**FUNCTIONS OF RETINAL GLIAL CELLS**

**Regulation of the Retinal Microenvironment**

One of the principal functions of retinal glial cells is to regulate the composition of the fluid in which neurons are bathed. Neuronal activity results in changes in neurotransmitter, K⁺, and H⁺ levels in the extracellular space, all of which can, in turn, influence subsequent neuronal excitability. Müller cells, and perhaps astrocytes as well, play a major role in regulating these extracellular constituents.

**Uptake of Neurotransmitters.** Synaptic transmission at glutamatergic synapses is terminated by the uptake of glutamate into neurons and glial cells. Müller cells and astrocytes have high-affinity glutamate transporters and play an important role in terminating synaptic transmission. Once glutamate is taken up by the glial cells, it is converted to glutamine and recycled back to the neuronal terminals, where it serves as a substrate for synthesis of additional neurotransmitter.

The glutamate transporter in glial cells may generate increases in extracellular glutamate levels under pathologic conditions. During anoxia and ischemia, [K⁺]i rises and pH₀ falls. An increase in extracellular K⁺ will slow down or even reverse glutamate transport, leading to an efflux of the transmitter from Müller cells and ultimately to excitotoxic damage to retinal neurons. The decrease in pH₀, accompanying ischemia, in contrast, will slow down the reverse transport of glutamate from Müller cells, thus protecting retinal neurons from excitotoxic damage.

Müller cells also have a high-affinity GABA transporter and are thought to participate in the termination of synaptic transmission at GABAergic synapses. GABA is converted to succinic semialdehyde once it is taken up by the glial cells.

**Regulation of Extracellular K⁺.** Müller cells play a key role in regulating [K⁺]₀ within the retina. Light-evoked neuronal activity results in increases in [K⁺]₀ in the inner and outer plexiform layers and to a [K⁺]₀ decrease in the distal retina. These [K⁺]₀ variations will change the membrane potential of retinal neurons, altering their excitability. The [K⁺]₀ variations are buffered by K⁺ currents flowing through Müller cells, a process termed K⁺ spatial buffering (Fig. 6-10). Excess K⁺ released from active neurons in the inner and outer plexiform layers leads to an influx of K⁺ into Müller cells in these regions and to the efflux of an equal quantity of K⁺ from other regions of the cell. Potassium is released from the basal endfoot into the vitreous humor, which acts as a large K⁺ sink, and into the subreti-
Fig. 6-11 Significance of Müller cell regulation of \([K^+]_o\). A, Light-evoked \([K^+]_o\) increases are recorded within the inner plexiform layer of the cat retina with a \(K^+\)-selective microelectrode. When Müller cell \(K^+\) channels are blocked by addition of 3 mM \(Ba^{2+}\), thus reducing the transfer of \(K^+\) to the vitreous humor, the light-evoked \([K^+]_o\) increase within the inner plexiform layer more than triples. B, Light-evoked \([K^+]_o\), decrease in the subretinal space. The decrease more than quadruples when \(Ba^{2+}\) is added, indicating that excess \(K^+\) is normally transferred to the subretinal space as well as to the vitreous humor. The time course of the light stimulus (4 seconds) is indicated at the bottom. (Modified from Frishman, LJ, Yamamoto, F, Bogucka, J, et al: J Neurophysiol 67:1201-1212, 1992.)

Fig. 6-12 Glial cell modulation of neuronal activity in the rat retina. For each trial (A to C), the spike activity of a neuron in the ganglion cell layer (top trace, plotted as a running average of spike frequency) and the \([Ca^{2+}]_o\) of glial cells adjacent to the neuron (bottom trace) are shown. A \(Ca^{2+}\) wave is initiated (arrow) by mechanical stimulation of a distant astrocyte. When the \(Ca^{2+}\) wave reaches the neuron (A and C), the spike activity of the neuron is inhibited. When the \(Ca^{2+}\) wave is initiated but fails to reach the neuron (B), there is no change in spike activity. (From Newman, EA, and Zahs, KR: J Neurosci 18:4022-4028, 1998.)

Regulation of Extracellular pH. The \(Na^+/HCO_3^-\) cotransport system of Müller cells and astrocytes is thought to influence \(pH_o\), within the retina. Neuronal activity results in the alkalization of the extracellular fluid in the distal retina and in the inner plexiform layer.\(^8,\)\(^124\) This alkalization can dramatically alter the efficacy of synaptic transmission and neuronal excitability.\(^2\) The magnitude of this alkalization may be reduced by the action of the glial cell cotransporter.\(^6\) The depolarization of glial cells induced by neuronal activity generates an acid efflux through the \(Na^+/HCO_3^-\) cotransporter, thereby countering the alkalization generated by the neurons.

Müller cells may also regulate \(pH_o\) within the retina through the action of carbonic anhydrase, which is expressed by Müller cells in both cytoplasmic and membrane-bound forms. Carbonic anhydrase contributes to \(pH_o\) buffering by catalyzing the conversion of \(H^+\) and \(HCO_3^-\) to \(CO_2\) and \(H_2O\). Blocking the action of the enzyme leads to increases in the light-evoked \(pH_o\) alkalization within the retina.\(^8\) Müller cells may also regulate retinal \(pH\) by aiding in the removal of \(CO_2\) from the retina. \(CO_2\) generated by neuronal activity diffuses freely into Müller cells, where it is converted by carbonic anhydrase into \(HCO_3^-\) and \(H^+\). These ions, in turn, may be transported to the vitreous humor through the action of the \(Na^+/HCO_3^-\) cotransporter and a \(Na^+/H^+\) exchanger.\(^68\)

Modulation of Neuronal Activity
As described in the preceding sections, Müller cells indirectly influence neuronal excitability by controlling the retinal microenvironment. Retinal glial cells may also modulate neu-
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Fig. 6-13 Glial cell induction of retinal vascularization. A and B, One quadrant of the surface of the developing rat retina. Both astrocytes (in red) and blood vessels (in green) migrate outward from the optic disc. (Superimposed astrocytes and blood vessels are in yellow.) B, The astrocytes migrate out ahead of the blood vessels and secrete vascular endothelial growth factor (VEGF) (in white) in response to hypoxic conditions. VEGF, in turn, induces further growth of the vessels. C to E, Cross section of the retina, with the nerve fiber layer at the top and the central retina off to the left. C, Blood vessels (in green) grow radially into the retina from the superficial vessels at the retinal surface. D, They grow along the radially oriented Müller cells (in red). E, Once they reach the inner nuclear layer, the blood vessels, stimulated by VEGF secretion from Müller cells (in white), grow within the inner nuclear layer. (From Stone, J. and Maslim, J. Prog Ret Eye Res 16:157-181, 1997.)

Glia activity directly. The light-evoked activity of retinal neurons, recorded in the ganglion cell layer, is altered when Ca \(^{2+}\) waves are propagated through glial cells at the retinal surface. Changes in neuronal spike activity occur only when these Ca \(^{2+}\) waves reach the neuron (Fig. 6-12). Both inhibition and excitation of spike activity occur. This modulation of neuronal excitability may be mediated by a Ca \(^{2+}\)-dependent release of glutamate from astrocytes or Müller cells, or both. This interaction between glial cells and neurons suggests that glial cells may directly participate in information processing in the retina.

Retinal Vascularization

Induction and Guidance of Retinal Blood Vessels. Glial cells induce the formation of blood vessels and guide their growth in the retina. During development, astrocytes migrate to the retina from the optic nerve, first appearing at the optic disc and then advancing outward across the retinal surface. Retinal blood vessels, which also originate from the optic nerve, grow along newly formed astrocyte processes, which function as templates for angiogenesis (Fig. 6-13, A and B). Vessel growth is stimulated by the secretion of vascular endothelial growth factor (VEGF), which is released by glial cells in response to hypoxic conditions generated by neuronal activity. Once vascular circulation has been established and normoxic conditions are achieved in a region, VEGF secretion is terminated, and further vessel formation in that region is halted. Astrocytes serve as guides for developing vessels as they grow across the inner surface of the retina, and Müller cells serve as templates for vessel growth into the retina and across the inner nuclear layer (Fig. 6-13, C to E).
**Induction of the Blood-Retinal Barrier.** Blood vessels within the retina and other parts of the CNS differ from vessels in other regions of the body in that they form a tight barrier that prevents the diffusion of ions and small molecules out of the vessels. This blood-retinal barrier is formed by tight junctions between the vascular endothelial cells of retinal blood vessels, junctions that are absent in vessels outside of the CNS. Glial cells induce the formation of these tight junctions. As blood vessels grow into the retina, their close association, first with astrocytes in the NFL, and then with Müller cells in deeper retinal layers, induce the formation of the endothelial cell junctions.

**Metabolic Support of Neurons**

Müller cells contain large reserves of glycogen that provide an energy source for retinal neurons. However, Müller cells and other glial cells do not function simply as passive conduits for glucose. There is a strong metabolic compartmentalization in the CNS, with glial cells and neurons each having specific metabolic functions and its own complement of enzymes. In the retina, Müller cells metabolize glucose to lactate, which is then transferred to neurons and which serves as their primary energy source for oxidative metabolism. In addition, the supply of lactate from glial cells to neurons can be regulated by neurotransmitters released from neurons.

**Generation of the Electroretinogram**

Müller cells generate several components of the electroretinogram (ERG), the light-evoked field potential recorded across the retina or the cornea. These potentials are generated by a radial current flow within the retina, established by K⁺ fluxes passing through Müller cells. The same K⁺-siphoning current that redistributes K⁺ within the retina (see Fig. 6-10) also generates the transretinal voltages recorded as components of the ERG.

**b-Wave.** The b-wave, a prominent corneal-positive ON component of the ERG, was until recently believed to be generated by Müller cells. Transient extracellular K⁺ increases in the outer and inner plexiform layers were thought to generate the b-wave current. However, recent analysis has suggested that Müller cells contribute little to b-wave generation. Rather, the response is generated primarily by bipolar cells, as was originally proposed long ago.

**Slow PIII Response.** The slow PIII is the retinal component of the c-wave response of the ERG. It is a corneal-negative response that is generated by K⁺ current flow through Müller cells, established by a decrease in [K⁺], in the distal retina. The same [K⁺], decrease generates a somewhat larger, corneal-positive response across the retinal pigment epithelium. The two responses, added together, comprise the corneal-positive c-wave.

**Proximal Retinal Responses.** Müller cells also generate several corneal-negative, proximal retinal responses. The scotopic threshold response is a rod-driven response generated at the ON of light in mammals. The M-wave is a negative response with prominent ON and OFF components and is recorded with intraretinal electrodes in amphibians. Both responses are generated by K⁺ current flow through Müller cells established by [K⁺], increases in the inner plexiform layer.

**SUMMARY**

The glial cells of the retina play a prominent role in retinal function. Both Müller cells and astrocytes have complex physiologic properties and possess a wide range of ion channels and receptors. They contribute to many essential retinal functions, including regulation of the retinal microenvironment, modulation of neuronal activity, control of developing retinal vessels, and active metabolic support of retinal neurons. They are an integral and essential element of the retina.

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