CHAPTER 7

Electrophysiology of Retinal Glial Cells

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CONTENTS

1. INTRODUCTION

The vertebrate retina, although developmentally part of the brain, is a unique central nervous system (CNS) structure in many respects. The glial cells of the retina are one example of this uniqueness. In most regions of the CNS, astrocytes and oligodendrocytes comprise the majority of glial cells. In the retina, the principal glial cell is the Müller cell. Astrocytes are found in the retinas of only mammalian species and oligodendrocytes are usually absent (the rabbit is one exception to this rule).

This review will briefly describe the morphology and proposed functions of retinal glial cells and then discuss in detail the electrophysiology of these cells. Possible functions of retinal glial cells suggested by electrophysiological results, principally, the regulation of K^+ levels in the retina and the generation of the electroretinogram (ERG), will then be discussed. Previous reviews of retinal glial cell structure and function include Ripps and Witkovsky (1985), Newman (1986a) and Schnitzer (1988a).

1.1. Morphology of Retinal Glial Cells

1.1.1. MÜLLER CELLS

Müller cells are radial glial cells (Ramon Y. Cajal, 1892). They extend from the vitreal surface of the retina to past the outer limiting membrane (Fig. 1). Müller cell somas are located in the mid to proximal (vitreal) portion of the inner nuclear layer. Projecting distally (towards the sclera) from the soma is a distal cell process which is terminated by microvilli which project into the subretinal space surrounding the photoreceptor outer segments. Projecting proximally from the soma is one or more proximal processes which terminate in endfeet. These endfeet lie at the vitreal surface of the retina and form the inner limiting membrane, a basal lamina (Pedler, 1963).

There is an extensive series of junctional complexes between Müller cells at the level of the outer limiting membrane. In reality, this structure is not a membrane but is given that appearance because of the junctions formed between adjacent Müller cells and between Müller cells and photoreceptors. In cold-blooded vertebrates, but apparently not mammals, Müller cells are coupled together by gap junctions (Dowling, 1970; Uga and Smelser, 1973).

Like astrocytes in other regions of the CNS, Müller cells send out fine cytoplasmic processes into the neural tissue of the retina (Ramon Y. Cajal, 1892). Most neuronal cell membrane is surrounded by these glial processes (Pedler, 1963; Miller and Dowling, 1970). The fine ramifications of Müller cells are particularly prevalent in the inner and outer plexiform layers. In the nerve fiber layer, Müller cell processes surround bundles of ganglion cell axons (Ogden, 1983). In the chick, these processes form myelin-like wrappings around optic fibers (Meller, 1984). In species with vascularized retinas, Müller cell processes surround retinal blood vessels (Kuwabara, 1969; Hogan *et al.*, 1971) and some, but not all, Müller cell endfeet terminate on vessels at the retinal surface (Drager *et al.*, 1984).

Müller cell morphology varies with retinal eccentricity. In rabbit, for instance, cells in the periphery of the retina are shorter, stouter and have a lower density (units: cells cm^{-2}) than do cells in the central retina (Reichenbach and Wohlrab, 1986).

1.1.2. ASTROCYTES

Astrocytes are found in the retinas of many mammals but are absent from the retinas of lower

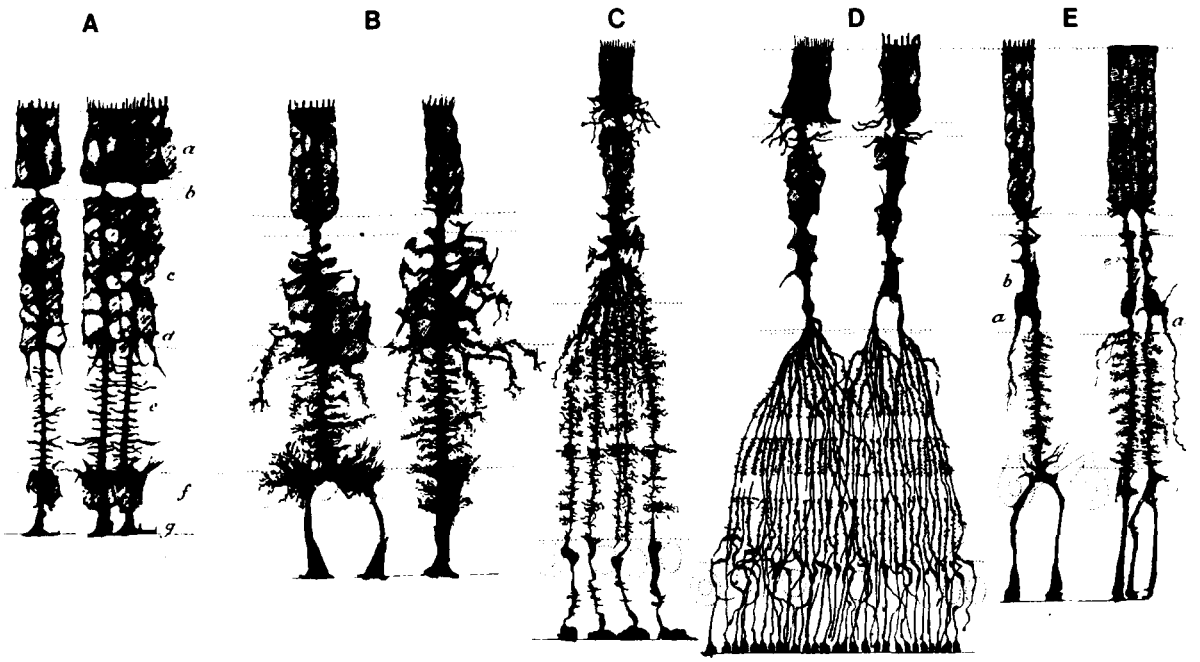

FIG. 1. Drawings of golgi-stained Müller cells by Cajal. From left to right are Müller cells of the frog (A), carp (B), lizard (C), chicken (D) and ox (E). Retinal layers are indicated in A as follows: a, outer nuclear layer; b, outer plexiform layer; c, inner nuclear layer; e, inner plexiform layer; f, ganglion cell layer; and g, inner limiting membrane. From Ramon Y. Cajal (1892).

vertebrates. In mammals, astrocytes are restricted to proximal layers of the retina (Karschin *et al.*, 1986; Schnitzer, 1988a). Most are localized to the nerve fiber layer where they are found in close association with ganglion cell axons. Some astrocytes are found in the ganglion cell layer and astrocyte processes sometimes project into more distal retinal layers. Astrocyte density is far lower than Müller cell density for a given species and retinal eccentricity (Drager *et al.*, 1984).

Astrocyte morphology varies with species and retinal eccentricity. These cells are usually classified as fibrous astrocytes. In the mouse and rat, astrocytes in all regions of the retina are stellate in form, having thin processes radiating from the soma (Drager *et al.*, 1984; Newman, unpublished observations). In cat, astrocytes in the periphery are also stellate. However, in the central region of the cat retina, where the nerve fiber layer is much thicker and many more ganglion cell fibers are found, astrocytes are bipolar in shape, with the cell processes running along side of nerve fibers (Karschin *et al.*, 1986). Astrocyte endfeet terminate on blood vessels at the retinal surface (Drager *et al.*, 1984).

1.1.3. OLIGODENDROCYTES

Axons within the retinas of almost all vertebrates are unmyelinated. Thus, oligodendrocytes, which form the myelin of axons within the CNS, are absent from the retina. An exception is the rabbit, which has medullary rays of myelinated axons which cover a small fraction of the retinal surface (Schnitzer, 1985).

1.2. Function of Müller Cells

Until recently, the functions of Müller cells in the retina, like those of astrocytes in the brain, were believed to lie largely in the domain of structural and metabolic support. However, work during the past two decades has indicated that these cells have other important retinal functions.

As discussed below, Müller cells are believed to regulate extracellular potassium levels, $[K^+]_o$, in the retina (Newman, 1985c). Müller cells are also thought to generate several components of the electroretinogram (Ripps and Witkovsky, 1985). Müller cells actively take up and degrade several putative retinal neurotransmitters, including glutamate (Ehinger, 1977), GABA (Sarthy, 1982) and acetylcholine (Sarthy and Lam, 1978). Thus, these cells probably play an active role in inactivating neurotransmitters after they are released from neuronal terminals. Müller cells contain high levels of carbonic anhydrase and most likely function to regulate CO_2 and pH levels in the retina (Sarthy and Lam, 1978). Müller cells also contain high levels of glycogen (particularly in species with avascular retinas) and undoubtedly help to meet the metabolic needs of the retina (Kuwabara and Cogan, 1961; Chase, 1982). Finally, Müller cells contain retinoid-binding proteins and may play a role in the visual cycle of synthesis and renewal of visual pigments in the retina (Bridges *et al.*, 1984).

2. MÜLLER CELL PHYSIOLOGY

2.1. Light Responses

Müller cell responses to light stimulation were first described by Miller and Dowling (1970) in the mudpuppy (Necturus). (Earlier recordings by Byzov [1965] in the Axolotl, attributed to "slow bipolars", were probably from Müller cells as well.) Müller cell responses have since been reported in a number of species, including salamander (Ambystoma: Miller *et al.*, 1977), frog (Rana: Mori *et al.*, 1976b; Xenopus: Witkovsky *et al.*, 1985), turtle (Conner *et al.*, 1985) and rabbit (Dick *et al.*, 1985). In general, the responses of all species resemble each other (see Fig. 2). Light stimuli of short duration (less than 1 sec) evoke slow Müller cell depolarizations which take up to a sec or more to peak. Responses take many seconds to decay. Longer duration light stimuli (i.e. 4 sec) elicit slow depolarizations at both ON and OFF of the stimulus (Fig. 2A). Prolonged light stimulation in dark-adapted eyes evoke a transient ON response

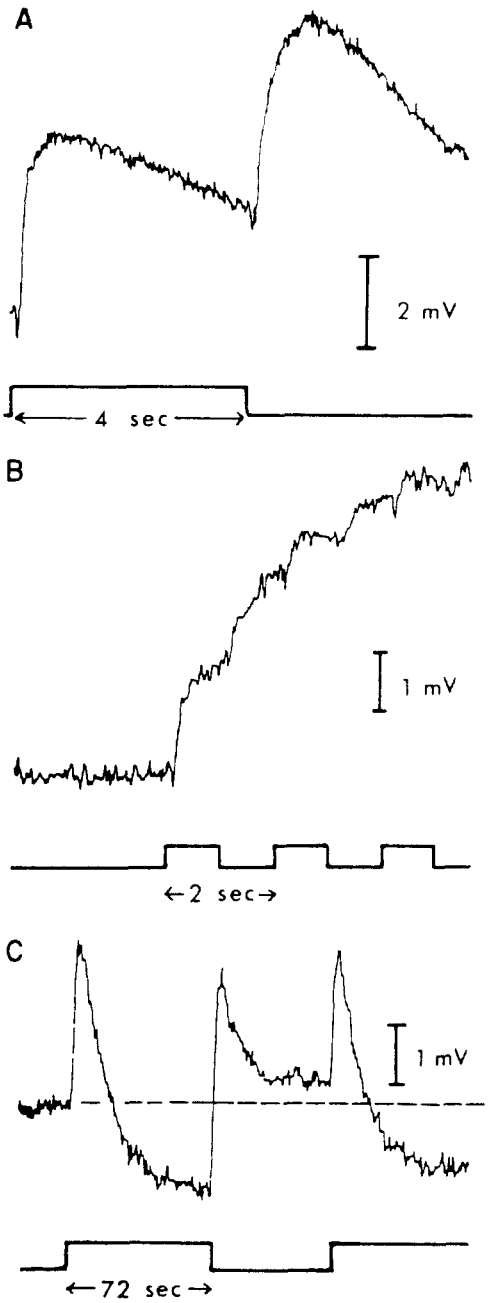

FIG. 2. Responses of Müller cells to visual stimulation, recorded from the mudpuppy eyecup (drained of vitreous humor). (A) A 4 sec presentation of a 0.5 mm spot evokes prominent ON and OFF responses. (B) Repetitive stimulation with a small spot evokes progressively smaller Müller cell responses. (C) A prolonged stimulus evokes an ON depolarization followed by a sustained hyperpolarization. From Karwoski and Proenza (1977).

followed by a sustained hyperpolarization (Fig. 2C; Karwoski and Proenza, 1977). The sustained hyperpolarization is more prominent in rabbit than in amphibians (Dick *et al.*, 1985). Müller cell light responses are seldom greater than 10 mV in amplitude and are typically much smaller.

A number of additional properties of Müller cell light responses have been described. Müller cells respond poorly to repetitive stimulation (Miller and Dowling, 1970; Karwoski and Proenza, 1977), as expected due to their slow response times. They give larger responses to small spot illumination (0.3 mm) than they do to large field illumination (Karwoski and Proenza, 1977; 1980a). In addition, Karwoski and Proenza (1977; 1980b) have described a transient response enhancement phenomenon in mudpuppy Müller cells. When a series of small spot stimuli are alternated with a large spot stimulus, the Müller cell response to the first small spot stimulus following a large spot presentation is significantly greater than previous small spot responses.

In their recordings from mudpuppy, Miller and Dowling (1970) demonstrated that Müller cell light responses resemble, in several respects, the *b*-wave of the ERG (Fig. 3). Both responses have similar latencies and time courses (at least for the rising phase of the responses). In addition the stimulus intensity vs response amplitude function for Müller cells is similar to that for the *b*-wave. Both responses have a larger dynamic range than do neuronal responses, which often saturate at lower stimulus intensities.

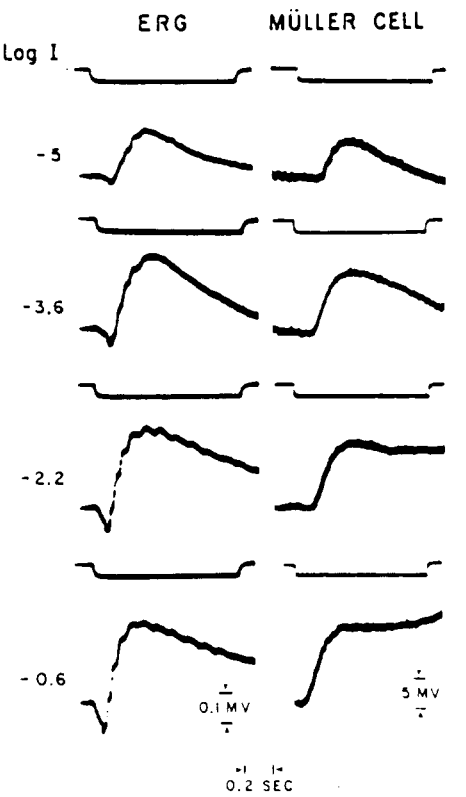

FIG. 3. ERG and Müller cell light responses evoked by flashes of four different intensities, recorded from the mudpuppy eyecup. The intracellular Müller cells response has a similar waveform as the transretinally recorded ERG *b*-wave, particularly at low stimulus intensities. At higher intensities, the Müller cell response remains depolarized for a longer time than does the *b*-wave (see Newman and Odette, 1984). In the ERG records, the positive-going *b*-wave follows the small, negative-going *a*-wave. From Miller and Dowling (1970).

2.2. Origin of Light Responses

The light responses of Müller cells are thought to be driven by light-evoked changes in extracellular K^+ concentration, $[K^+]_o$ (Karwoski and Proenza, 1977). As first demonstrated by Kuffler and his colleagues (Kuffler, 1967), astrocytes in the CNS depolarize in response to neuronal activity. These depolarizations are driven by the release of K^+ from active neurons. Similarly, the depolarizations and hyperpolarizations of retinal Müller cells arise from the release or uptake of K^+ from active neurons in the retina. Müller cell input resistance does not change during light responses, consistent with the notion that the responses are driven by variations in $[K^+]_o$ rather than by changes in cell conductance (Nelson, 1973).

Ion-selective microelectrode measurements of light-evoked variations in $[K^+]_o$ have confirmed that Müller cell responses are closely correlated with $[K^+]_o$ variations in the retina (Karwoski and Proenza, 1977; 1980a). At light onset, there is a relatively large, slow increase in $[K^+]_o$ in the proximal portion of the inner plexiform layer and a smaller, more transient $[K^+]_o$ increase in the outer plexiform layer (Kline *et al.*, 1978; 1985; Dick and Miller, 1978; 1985; Dick *et al.*, 1985;

Karwoski and Proenza, 1978). These $[K^+]_o$ increases lead to the ON response of the Müller cell. At light offset, a second $[K^+]_o$ increase occurs in the distal half of the inner plexiform layer, leading to the Müller cell OFF response. Measurements in retinal slices demonstrate that the light-evoked $[K^+]_o$ increases are generated almost exclusively in the two plexiform layers (Karwoski *et al.*, 1985).

In the dark adapted retina, prolonged light stimulation leads to a gradual decrease in $[K^+]_o$. This $[K^+]_o$ decrease is generated by a K^+ sink at the level of the rod inner segments. The $[K^+]_o$ decrease diffuses proximally into the neural retina (Oakley and Green, 1976; Karwoski and Proenza, 1978; Steinberg *et al.*, 1980) and hyperpolarizes Müller cells.

2.3. Other Responses

Müller cells depolarize under a number of experimental conditions which result in increases in retinal $[K^+]_o$. Activation of ganglion cells by antidromic stimulation of the optic nerve evoke small, slow depolarizations in Müller cells (Miller *et al.*, 1977). Similarly, direct application of K^+ onto the retina leads to Müller cell depolarization (Newman, 1985a). Spreading depression episodes in the retina are accompanied by large increases in $[K^+]_o$ arising from the inner plexiform layer (Mori *et al.*, 1976a; 1976b). These $[K^+]_o$ increases are accompanied by large Müller cell depolarizations (Mori *et al.*, 1976a; 1976b).

2.4. Cell Coupling

The existence of gap junctions between Müller cells of cold-blooded vertebrates suggests that these cells, like CNS astrocytes, may be electrically coupled (Dowling, 1970). This has proved to be the case. Recordings from the turtle (Conner *et al.*, 1985), and axolotl (Attwell *et al.*, 1985) retina have confirmed that Müller cells are electrically coupled, albeit weakly. Attwell *et al.* (1985) have suggested that electrical coupling in Müller cells may mediate lateral spatial buffering of K^+ within the retina (see Section 4.1.). However, Gardner-Medwin (1986) has pointed out that because the coupling is so weak, lateral spatial buffering is of little consequence in the regulation of $[K^+]_o$ in the retina.

3. MÜLLER CELL MEMBRANE PROPERTIES

A great deal has been learned about the membrane properties of Müller cells within the past decade. Much of this work has utilized the dissociated cell preparation. Freshly isolated Müller cells, obtained from enzymatically treated retinas, are easily recorded from with conventional or suction electrodes. They can be voltage-clamped and single-channel currents can be monitored. It must be borne in mind, however, that dissociated cells, even freshly isolated ones, may not have identical membrane properties as *in situ* cells.

3.1. Resting Potential

The membrane potential of Müller cells *in situ* is approximately -80 to -90 mV, much larger than the membrane potential of retinal neurons. Resting potential values of -93 mV in the frog ($[K^+]_o$ = 2.5 mM; Newman 1985a), -77 mV in the mudpuppy ($[K^+]_o$ = 2.5 mM; Shimazaki *et al.*, 1984), -72 mV in the turtle ($[K^+]_o$ = 3.3 mM; Conner *et al.*, 1985), and approximately -85 mV in the rabbit ($[K^+]_o$ = 5 mM; Dick *et al.*, 1985) have been reported. These large values suggest that the membrane potential is dominated by the K^+ equilibrium potential of the cell (Section 3.2).

3.2. Ion Selectivity

Like astrocytes and oligodendrocytes (Ransom and Carlini, 1986; Kettenmann *et al.*, 1983), Müller cells are almost exclusively permeable to K^+. When $[K^+]_o$ is varied experimentally, the Müller cell membrane potential faithfully follows the predicted K^+ equilibrium potential at all but the lowest $[K^+]_o$ values (Newman, 1985a; Conner *et al.*, 1985). In frog Müller cells *in situ* (Newman, 1985a) the cell membrane potential deviates from the K^+ potential only at $[K^+]_o$ values below

2.5 mM. This deviation is abolished if choline is substituted for Na^+ in the extracellular medium, suggesting that the residual membrane conductance (in addition to the K^+ conductance) is a Na^+ conductance. The estimated K^+: Na^+ permeability ratio in *in situ* frog Müller cells is 490 (Newman, 1985a).

In dissociated cells, estimated K^+: Na^+ permeability ratios are somewhat lower. Permeability ratios of 27 in turtle (Conner *et al.*, 1985), 75 in salamander, 73 in rabbit and 114 in monkey (Newman, 1987) have been measured. It is possible that the procedures used in isolating single cells, i.e. papain digestion, may make the Müller cell membrane somewhat less selective to K^+ (Newman, 1987).

3.3. Distribution of K^+ Conductance in Amphibians

Newman (1984) has demonstrated that the K^+ conductance of Müller cells is not distributed uniformly over the cell surface but rather is localized to specific cell regions. Several different types of observations from a number of laboratories have confirmed this finding.

3.3.1. CELL IMPEDANCE

In a series of experiments on freshly dissociated salamander Müller cells, Newman (1984, 1985a) has shown that cell input impedance is, normally, approximately 8.5 MΩ. If, however, the endfoot of an isolated call is removed by cutting it off with a glass needle, the impedance of the remainder of the cell rises to approximately 150 MΩ. A similar, high impedance is measured in cells whose endfeet are broken off during the dissociation procedure. The change in impedance indicates that 95% of the total conductance of the cell is localized to the endfoot. Based on impedance measurements, Newman (1985a) has estimated that the endfoot of salamander Müller cells has a specific membrane resistance of 32 $\Omega \cdot cm^2$ while the resistance of the soma is 7300 $\Omega \cdot cm^2$. The specific capacitance of the membrane of dissociated salamander cells is approximately 1.77 $\mu F \cdot cm^{-2}$ (Newman, 1985a).

3.3.2. K^+ EJECTION RESPONSES

Newman (1984, 1985a) confirmed the existence of high membrane conductance in the endfoot of salamander Müller cells by monitoring the responses of dissociated cells to localized increases in $[K^+]_o$. An increase in $[K^+]_o$ at the endfoot (produced by pressure ejecting a high K^+ solution from a micropipette) evokes much larger cell depolarizations than does $[K^+]_o$ increases in other cell regions (Fig. 4). The magnitude of a response to a local $[K^+]_o$ increase is directly proportional to the K^+ conductance of the membrane experiencing the $[K^+]_o$ increase (Newman, 1985a). Thus, the K^+ ejection experiment demonstrates that the endfoot of salamander Müller cells has a much larger K^+ conductance than do other cell regions.

3.3.3. SINGLE CHANNEL RECORDINGS

Brew *et al.* (1986) have measured the density of K^+ channels across the surface of dissociated salamander Müller cells using patch clamp techniques. They report that the density of K^+ channels (K^+ inward rectifiers) is much greater on the endfoot surface than on the soma. Nilius and Reichenbach (1988), in contrast, report that several different types of ion channels occur in dissociated rabbit Müller cells (Section 3.6). The channels found on the endfeet of these cells have a significantly higher unitary conductance than do the channels found in other cell regions.

3.3.4. ELECTROTONIC MEASUREMENTS

Winslow *et al.* (1987) have estimated the distribution of K^+ conductance in dissociated salamander Müller cells by analyzing the time course of the charging curve of cells depolarized by current injection. Their results suggest that approximately 95% of the cell conductance is localized to the endfoot.

3.3.5. FIELD POTENTIALS

As is discussed in detail below (Section 5.) K^+ current flow through Müller cells *in situ* is thought to generate field potentials within the retina. The

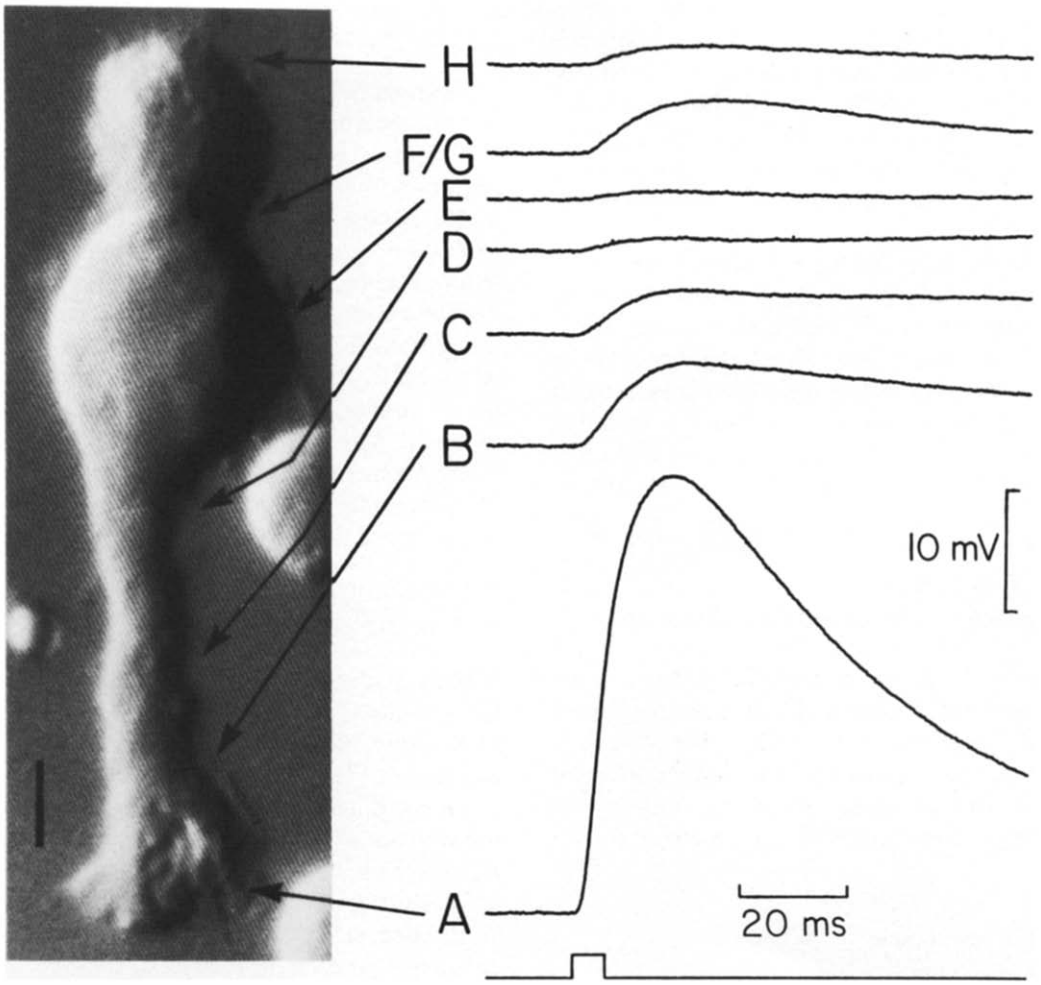

FIG. 4. Voltage responses of a dissociated salamander Müller cell to focal K^+ ejections. Recordings were made from the cell shown at left. Potassium ejection onto the endfoot (A) evokes a much larger cell depolarization than do ejections onto other cell regions (B through H). Such responses indicate that the endfeet of Müller cells have a much greater K^+ conductance than do other regions of the cell. Scale bar in photograph, 10 μm. From Newman (1987).

intraretinal distribution of these potentials is consistent with Müller cell endfeet having high K^+ conductance. Field potentials generated during spreading depression episodes have a vitreal positive polarity (Mori *et al.*, 1976a,c). They are believed to arise from K^+ entering Müller cells in the inner plexiform layer and exiting from near the surface of the retina (the endfoot region; Mori *et al.* 1976a). Similarly, K^+ ejected from a micropipette within the retina leads to the generation of a vitreal positive transretinal potential for almost all ejection locations within the retina (Fujimoto and Tomita, 1981; Yanagida and Tomita, 1982). Finally, current-source density analysis of the *b*-wave of the ERG in the frog indicates that a current influx into Müller cells in both the inner and outer plexiform layers is balanced by current efflux largely from the endfoot (Newman, 1979; 1980). All of these observations can be explained most simply if the endfeet of Müller cells have a much larger membrane conductance than other regions of the cell.

3.4. Distribution of K^+ Conductance in Mammals

The distribution of K^+ conductance across the surface of mammalian Müller cells has been

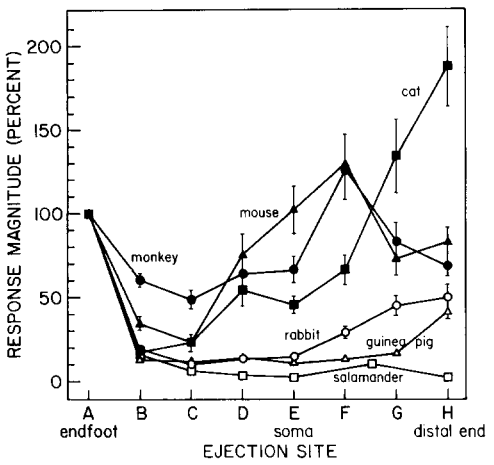

FIG. 5. Summary graph of voltage responses evoked by K^+ ejections recorded from Müller cells of the tiger salamander (□), rabbit (○), guinea pig (△), mouse (▲), owl monkey (●), and cat (■). Responses are expressed as a percentage of the endfoot responses, which are normalized to 100%. In Müller cells of species with avascular retinas (salamander, rabbit, guinea pig), endfoot responses (and hence endfoot conductance) are much greater than responses of other cell regions. In cells of vascularized species (mouse, monkey, cat), cell responses (and cell conductance) are largest on the soma or along the distal cell process. From Newman (1987).

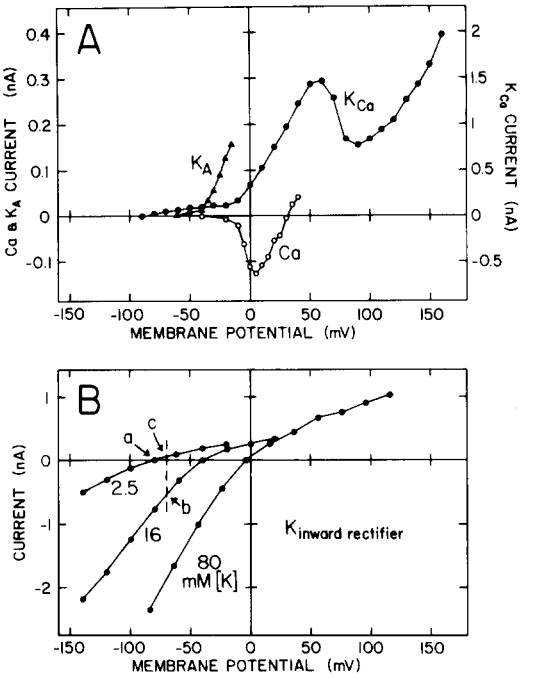

FIG. 6. Current-voltage relations of four voltage-dependent currents recorded from dissociated salamander Müller cells. These plots were obtained in whole-cell voltage clamp experiments using patch electrodes. (A) Inward Ca^{2+} currents (Ca) and outward Ca^{2+}-activated K^+ currents (K_{Ca}) are observed when cells are depolarized to past -40 mV. Type-A K^+ currents (K_A) are observed for depolarizations past -60 mV. (B) Inward rectifying K^+ currents are recorded from a cell bathed sequentially in 2.5, 16 and 80 mM K^+ perfusate. From Newman (1985b).

determined by Newman (1987) using the K^+ ejection response technique. Results demonstrate that, as in amphibians, K^+ conductance is localized to specific regions of the cell (Fig. 5). However, the pattern of K^+ conductance is more complex in mammals than it is in amphibians. In rabbit and guinea pig, which both have avascular retinas, K^+ conductance is much higher in the endfoot than in other cell regions. The distribution of K^+ conductance in these two species is similar to that of the salamander, which also has an avascular retina. Reichenbach (personal communication) has confirmed this finding for the rabbit.

In mouse, monkey and cat, on the other hand, which all have vascularized retinas, K^+ conductance is highest in the soma or in the distal cell process. Newman (1987) has suggested that the high K^+ conductance of Müller cells in vascularized mammalian species may be associated with cell processes in contact with retinal capillaries. If this proves to be the case, then the processes of Müller cells in contact with blood vessels should probably be considered to be endfeet.

3.5. Voltage-Dependent Conductances

A voltage-clamp study of dissociated salamander Müller cells by Newman (1985b) has demonstrated the existence of four voltage-dependent conductances in these cells (Fig. 6). Three conductances turn on when cells are depolarized beyond approximately −40 mV, a voltage-dependent Ca^{2+} conductance, a Ca^{2+}- and voltage-dependent K^+ conductance, and a fast-inactivating (type A) K^+ conductance. A fourth voltage-dependent conductance, a K^+ inward rectifier, is open at the cell rest potential.

Newman (1985b) also indicated that the large K^+ conductance at the cell endfoot is of a fifth type and is largely voltage-independent.

In the same study (Newman, 1985b), regenerative Ca^{2+} spikes were recorded from salamander Müller cells *in situ*, demonstrating that the observed Ca^{2+} conductance did not arise as a consequence of the dissociation procedure. Voltage-dependent Na^+ currents were not observed.

Conner *et al.* (1985) have reported inward rectifying properties in dissociated turtle Müller cells. Veratridine-activated tetrodotoxin-inhibited Na^+ channels may also occur in Müller cells (Sarthy, 1983).

3.6. Ion Channels

A patch-clamp study by Brew *et al.* (1986) has revealed inward rectifying K^+ channels on both the cell soma and endfoot of dissociated salamander Müller cells. The authors find that channel density is much higher on the endfoot than on the soma and suggest that the high conductance of Müller cell endfeet is due to a high density of inward rectifying channels on that structure. However, this conflicts with Newman's (1985b) observation that endfoot conductance is largely voltage-independent in salamander Müller cells.

In dissociated rabbit Müller cells, Nilius and Reichenbach (1988) report the existence of several types of K^+ ion channels. High conductance, strongly inward rectifying K^+ channels are found on the soma and proximal process of isolated cells. In contrast, non-rectifying, "maxi" conductance channels are found on the cell endfoot. Low conductance, weakly-rectifying K^+ channels are also found in other cell regions.

3.7. Neurotransmitters

Müller cells actively take up several putative retinal neurotransmitters, including glutamate (Ehinger, 1977; Brandon and Lam, 1983), GABA (Neal and Iversen, 1972; Sarthy, 1982), and Taurine (Ehinger, 1973; Schulze and Neuhoff, 1983).

Henshel and Miller (1986) have studied the physiological effects of glutamate on dissociated salamander Müller cells. Application of micromolar concentrations of glutamate depolarize Müller cells. Under voltage-clamp conditions, glutamate application results in an inward current which is decreased by removal of external Na^+. Responses of cells to glutamate agonists and antagonists suggests that the inward current is generated by a high affinity glutamate uptake system.

Brew and Attwell (1987) have also studied the effects of glutamate on dissociated, voltage-clamped salamander Müller cells. Application of glutamate generates an inward current which is abolished, but not reversed, when Na^+ is removed from the external solution. The glutamate-induced current is reduced by the glutamate uptake blocker *p*-chloromercuriphenylsulphonate, indicating that the inward current is generated by an electrogenic glutamate transporter. The authors suggest that glutamate-induced currents in Müller cells may contribute to the generation of the ERG *b*-wave. However, their hypothesis cannot account for the generation of inward *b*-wave currents at the level of the outer plexiform layer (Newman, 1980).

The effects of putative retinal transmitters and antagonists on Müller cell responses have been studied in the amphibian retina. L-Alpha aminoadipic acid preferentially abolishes the ON component of the Müller cell light response while D-alpha-aminoadipic acid preferentially reduces the OFF component (Zimmerman and Corfman, 1984). Aspartate reduces or abolishes both Müller cell ON and OFF responses (Shimazaki *et al.*, 1984). 2-amino-4-phosphonobutyric acid blocks the Müller cell ON response (Zimmerman and Corfman, 1984; Witkovsky *et al.*, 1985). Picrotoxin enhances both ON and OFF responses and is antagonized by GABA, which suppresses the light response (Witkovsky *et al.*, 1985). Taurine and glycine both block Müller cell OFF responses and reduce ON responses (Cunningham and Miller, 1980). The actions of these agents are presumably due to their effects on neuronal synapses and are not due to direct action on Müller cells. Alpha-aminoadipic acid is also a gliotoxic agent and can damage Müller cells (Pedersen and Karlsen, 1979; Szamier *et al.*, 1981).

3.8. Pharmacology

The pharmacology of the ionic conductances of Müller cells have been studied in voltage-clamp studies of dissociated salamander cells (Newman, 1985b). Calcium currents are blocked by Cd^{2+} (1 – 2 mM) and verapamil (50 μM), Ca^{2+}-activated K^+ currents by tetraethylammonium (1 mM), and fast-inactivating K^+ currents by 4-aminopyridine (5 mM).

Barium, in micromolar concentrations (40 μM) blocks more than 90% of the K^+ conductance of dissociated salamander Müller cells (Newman, 1985b; Henshel *et al.*, 1985). However, Ba^{2+}, even in mM concentrations, has little or no effect on Müller cell conductance *in situ* (Newman, 1985b; Henshel *et al.*, 1985). The explanation for this inconsistency is not known, although it suggests that the papain digestion used in preparing dissociated cells may be altering K^+ channel properties.

The action of Ba^{2+} on Müller cells is further complicated by its affects on the ERG. Müller cells, in response to light-evoked changes in $[K^+]_o$, are thought to generate both the *b*-wave of the ERG and the slow PIII response (see Section 5). However, Ba^{2+}, when added to the retina of the rat (Winkler and Gum, 1981) or salamander (Coleman *et al.*, 1987), blocks the slow PIII response while having little effect on the *b*-wave. These results call into question the Müller cell origin of the *b*-wave response. However, Coleman *et al.* (1987) suggest that Ba^{2+}, in its action on the standing $[K^+]_o$ gradient within the retina, may bias Müller cell inward rectifying channels so that they do not respond to the $[K^+]_o$ decrease which generates the slow PIII response but continue to respond to the *b*-wave $[K^+]_o$ increase.

3.9. Na^+,K^+-ATPase

The Na^+,K^+-ATPase inhibitor ouabain, when added to the retina, causes a rapid Müller cell depolarization of several mV (Newman, 1985a; Reichenbach *et al.*, 1987), indicating the existence of an electrogenic Na^+, K^+ pump in these cells. Reichenbach and colleagues have investigated the ionic dependency of the pump in dissociated rabbit Müller cells. Pump activity is enhanced as $[K^+]_o$ is increased up to 10 mM (Reichenbach *et al.*, 1985). In addition, high pump activity continues in low-Na^+ or Na^+-free solutions (Reichenbach *et al.*, 1987), suggesting that internal Na^+ is not required for pump activity. These ionic properties differ from those of the standard neuronal Na^+,K^+-ATPase, which is not enhanced by $[K^+]_o$ increases above 3 mM and which is strongly dependent on internal $[Na^+]$. (Using immunohistochemical methods, McGrail and Sweadner (1986) have shown that the Na^+,K^+-ATPase in rat Müller cells is distinct from that found on some retinal neurons.) The $[K^+]_o$ dependency of the Müller cell Na^+,K^+-ATPase is well suited to aid in the regulation of $[K^+]_o$ in the retina by an active uptake process (Reichenbach *et al.*, 1985).

Electrogenic pump activity is evident in dissociated rabbit Müller cells exposed to high $[K^+]_o$ solutions (Reichenbach and Eberhardt, 1986; Reichenback *et al.*, 1986). The initial depolarization in response to a high $[K^+]_o$ solution is followed by a marked repolarization and, following removal of high $[K^+]_o$, a transient hyperpolarization occurs. Both the repolarization and the after hyperpolarization are eliminated by ouabain treatment. In frog Müller cells *in situ* (Newman, 1985a), however, exposure to high $[K^+]_o$ is not accompanied by either repolarization or by an after hyperpolarization.

4. REGULATION OF RETINAL K^+ LEVELS

Müller cells may help to regulate $[K^+]_o$ in the retina by several mechanisms (see Gardner-Medwin, 1983). The demonstration that the Na^+, K^+-ATPase in rabbit Müller cells is enhanced by $[K^+]_o$ up to 10 mM (Reichenbach *et al.*, 1985) suggests that active uptake by Müller cells contributes to $[K^+]_o$ regulation, at least in mammals. Passive K^+ uptake, the influx of K^+ into Müller cells along with a counter ion, may also help to regulate $[K^+]_o$. Finally K^+ siphoning, the flow of K^+ current into and out of Müller cells, may be an important regulatory mechanism.

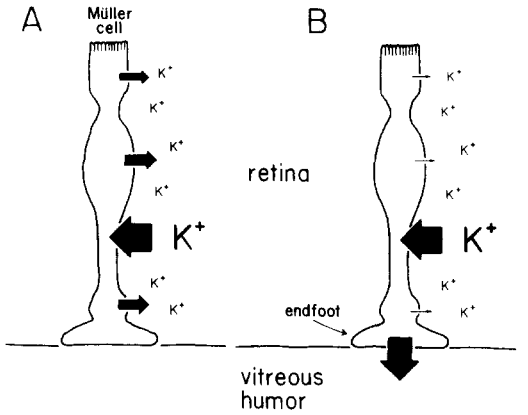

FIG. 7. Diagrams illustrating the process of K^+ siphoning by Müller cells in the retina. (A) If K^+ conductance was distributed uniformly over the cell surface, an increase in $[K^+]_o$ in the inner plexiform layer would cause a K^+ influx into Müller cells in this region (large arrow) and a K^+ efflux from all other regions of the cell (small arrows). (B) Because most of the conductance of the Müller cell is localized to the endfoot process, almost all K^+ efflux will occur from this region. Excess K^+ from the inner plexiform layer will be transferred to the vitreous humor rather than to other retinal layers, as in (A). From Newman (1986c).

4.1. K^+ Siphoning

Orkand, Nicholls and Kuffler (1966) suggested that astrocytes may regulate $[K^+]_o$ in the brain by K^+ spatial buffering. Potassium flows into glial cells in regions where $[K^+]_o$ is raised by neuronal activity. This K^+ influx depolarizes the cells and drives an equal amount of current out from other cell regions, or from electrically coupled cells. Because glial cells are almost exclusively permeable to K^+, this current efflux will also be carried by K^+. The net effect of this current flow is to transfer K^+ from regions where $[K^+]_o$ is high to regions where $[K^+]_o$ is low.

As suggested by Newman (1984; 1985c) a similar process will take place in the retina. Light evoked increases in $[K^+]_o$ will cause an influx of K^+ into Müller cells, primarily in the inner and outer plexiform layers. If K^+ conductance were distributed uniformly over the surface of Müller cells, the K^+ influx would be balanced by a K^+ efflux from all other regions of the cell. These spatial buffer currents would help to redistribute the initial $[K^+]_o$ increases, but only at a cost. The initial increases in $[K^+]_o$ in the plexiform layers would be reduced by raising $[K^+]_o$ elsewhere in the retina (Fig. 7A).

However, as summarized in Sections 3.4 and 3.5, K^+ conductance is not distributed uniformly in Müller cells. In amphibians and in mammals with avascular retinas, a large fraction of the total cell K^+ conductance is localized to the endfoot (Newman, 1987). Thus, the K^+ influx in the plexiform layers will be balanced by a K^+ efflux primarily from the high conductance endfoot (Fig. 7B). The K^+ released from the endfoot will, in turn, diffuse into the vitreous humor.

This directed flow of spatial buffer current through the endfoot of Müller cells is termed 'K^+ siphoning' (Newman *et al.*, 1984). Its principal advantage over the spatial buffer scheme of Orkand *et al.* (1966) is that excess K^+ is directed to the vitreous humor, which acts as a large reservoir or sink of K^+, instead of into adjacent neural tissue.

The distribution of K^+ conductance in Müller cells of mammals having vascularized retinas is more complex and, in these species, the K^+ siphoning story is less clear. In mouse and monkey, the endfoot has higher K^+ conductance than some other cell regions. Thus, a portion of the K^+ spatial buffer current will be shunted out through the endfoot. In these species, however, the highest K^+ conductance lies within the inner nuclear layer. Newman (1987) has suggested that this high conductance may be associated with Müller cell processes in contact with blood vessels. If so, a fraction of the excess K^+ which enters Müller cells will be siphoned out directly onto retinal vessels. It is possible, but not established, that this K^+ is actively transported into the blood.

Newman *et al.* (1984) have obtained direct confirmation that K^+ siphoning occurs in salamander Müller cells (Fig. 8). They measured K^+ efflux from dissociated cells using K^+-selective micropipettes. When the distal (photoreceptor) end of a cell is exposed to high $[K^+]_o$, K^+ efflux is detected only from the cell endfoot.

4.2. Effectiveness of K^+ Siphoning

Newman *et al.* (1984) evaluated the effectiveness of K^+ siphoning in regulating $[K^+]_o$

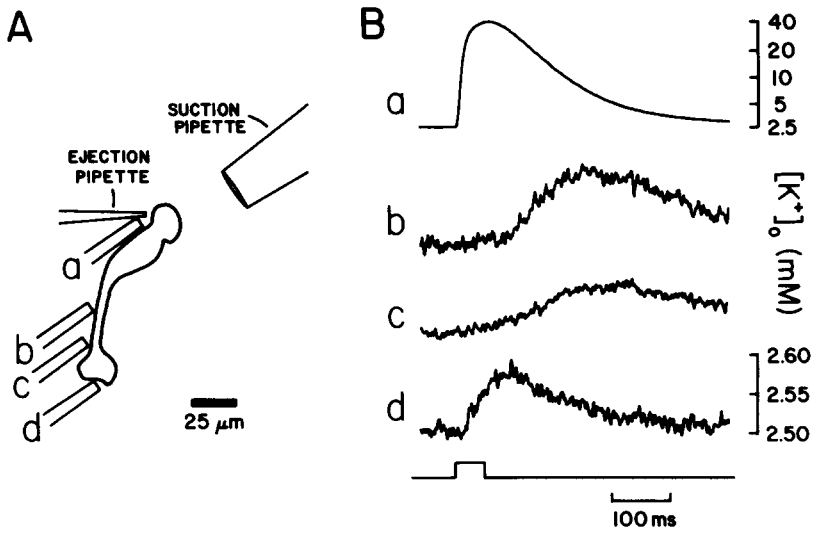

FIG. 8. Experimental demonstration of K^+ siphoning. (A) The apical (photoreceptor) end of a dissociated salamander Müller cell is exposed to increased $[K^+]_o$ by ejecting an 85 mM KCl solution from a pipette. Efflux of K^+ from other regions of the cell is monitored with a K^+-selective microelectrode (a through d). (B) Responses recorded from the K^+-selective electrode. K^+ efflux from the cell is only detected at site d, the proximal face of the endfoot. Traces b, c and d are expanded vertically relative to trace a. From Newman *et al.* (1984).

using a numerical computer model of K^+ dynamics in the retina. They found that for a 1 mM increase in $[K^+]_o$ in the inner plexiform layer, siphoning of K^+ into the vitreous humor is several times more effective in clearing the excess K^+ than is diffusion of K^+ through extracellular space; K^+ siphoning has a half-clearance time of 2.1 sec vs a half-clearance time of 7.7 sec for diffusion.

Gardner-Medwin (1986) has evaluated the effectiveness of various clearance mechanisms using the concept of a "buffer capacity" for each mechanism. He concludes that in the retina, K^+ siphoning may be significantly more important than K^+ diffusion as a clearance mechanism. However, he suggests that under most conditions, uptake of K^+ into cytoplasmic pools may be more important than K^+ siphoning in clearing K^+ from extracellular space.

Brew and Attwell (1985), in another theoretical treatment of K^+ siphoning, found that a maximal amount of K^+ could be transported from the retina to the vitreous by siphoning currents if 50%, rather than 95% of total cell conductance (the case for salamander) is localized to the endfoot. The endfoot conductance of Müller cells of avascular mammalian retinas is significantly less than 95% of total cell conductance (Newman, 1987) and appears to be more in line with Brew and Attwell's (1985) prediction. It must be remembered, however, that one of the advantages of K^+ siphoning is that it results in almost all of the excess K^+ within the retina being transported to the vitreous. This advantage is lost as endfoot conductance is lowered.

Reichenbach and Wohlrab (1986) have demonstrated that Müller cell morphology varies greatly with retinal eccentricity in the rabbit and may have an important influence on K^+ siphoning. Specifically, the proximal process of Müller cells in the central retina is long and narrow, giving it a high longitudinal resistance. This high resistance limits the flow of spatial buffer currents in Müller cells and thus, compromises the rate at which K^+ is transported by these cells. Eberhardt and Reichenbach (1987) have evaluated this phenomenon in an analytical model of K^+ clearance in the retina and find that the effectiveness of K^+ siphoning as a clearance mechanism, as compared to K^+ diffusion through

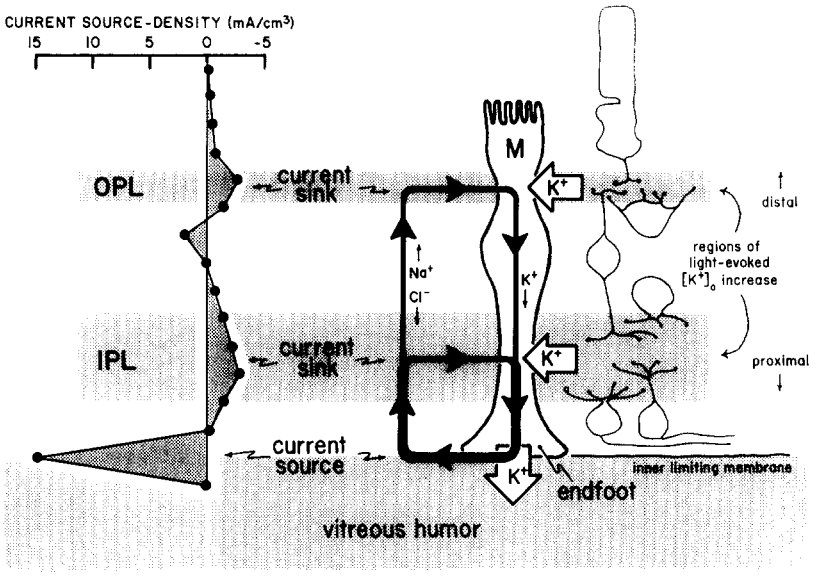

FIG. 9. Generation of the ERG *b*-wave by Müller cells. The results of a current source-density analysis of the *b*-wave (shown at left) demonstrate that the ERG response is generated by two current sinks in the inner and outer plexiform layers and by a current source at the vitreal surface of the retina (data from a frog eyecup; Newman, 1980). The current sinks arise as K^+ current enters the cell in regions of light-evoked increase in $[K^+]_o$ (indicated by open arrows at right of figure). Almost all of this current exits from the endfoot of the Müller cell. This efflux is recorded as a current source. The current flow through extracellular space established by these K^+ fluxes (solid lines) generates the *b*-wave. This pattern of current flow also leads to the transfer of excess K^+ from the plexiform layers to the vitreous humor. M, Müller cell; OPL, outer plexiform layer; IPL, inner plexiform layer. From Newman (1985c).

extracellular space, is greatest for Müller cells having a length of approximately 110 μm, near the median length of Müller cells in the rabbit retina.

5. GENERATION OF FIELD POTENTIALS

As detailed above, light-evoked increases in $[K^+]_o$ in the two plexiform layers of the retina lead to influxes of K^+ into Müller cells. This current exits from other regions of the cell, principally from the endfoot in species with avascular retinas. The return current flow through extracellular space leads to the generation of transretinal potentials. The time course and distribution of these potentials within the retina are dependent on the pattern of the initial $[K^+]_o$ increases. Many components of the ERG, including the *b*-wave, the slow PIII response, the *M*-wave, the scotopic threshold response and a portion of the pattern ERG are believed to be generated in this manner. These ERG components are, in essence, epiphenomena arising from the process of K^+ spatial buffering.

5.1. ERG *b*-Wave

The ERG *b*-wave is the best studied field potential thought to be generated by Müller cells. Miller and Dowling (1970) found that light responses of Müller cells in the mudpuppy closely resemble the *b*-wave of the ERG. A current source-density study by Faber (1969) in the rabbit also suggests a Müller cell origin of the *b*-wave response. These workers proposed that radial current flow through Müller cells, generated by light-evoked $[K^+]_o$ increases in the distal retina, generate the *b*-wave.

Many lines of evidence support this Müller cell hypothesis of *b*-wave generation. The surface area of Müller cells within the outer plexiform layer increases markedly at the same developmental stage that the *b*-wave first appears (Rager, 1979).

Newman (1979, 1980) has shown that the two principal sinks of current which generate the *b*-wave have similar time courses and locations within the retina as the two light-evoked $[K^+]_o$ increases recorded with K^+-selective microelectrodes (Fig. 9). In addition, the principal *b*-wave current source is located at the surface of the retina, the location of the Müller cell endfoot.

Current-source density measurements indicate that, in the frog, 65% of the peak *b*-wave response is generated by the $[K^+]_o$ increase in the outer plexiform layer (Newman, 1980; Newman and Odette, 1984). This finding is in agreement with pharmacological studies in mudpuppy (Dick and Miller 1978, 1985) and rabbit (Dick *et al.*, 1985) which show that agents that augment the light-evoked $[K^+]_o$ increase in the outer plexiform layer enhance the *b*-wave response. In addition, raising $[K^+]_o$ within the retina, which reduces light-evoked K^+ influx into Müller cells, also reduces the *b*-wave response (Miller, 1973).

The distribution of K^+ conductance in Müller cells of mammals with vascularized retinas is quite different from that of other vertebrates (Newman, 1987), and the pattern of K^+ induced current flow should be correspondingly different. For instance, a $[K^+]_o$ increase in the inner plexiform layer in an amphibian should generate a current which exits the Müller cell principally from the endfoot (Mori *et al.*, 1976a; Newman, 1980; Fujimoto and Tomita, 1981). Consequently, a vitreal positive potential is generated. In a monkey or cat, however, K^+ influx in the inner plexiform layer should induce a K^+ efflux primarily in the distal portion of the Müller cell, resulting in a vitreal negative potential (see Kline *et al.*, 1978). Thus, the distribution of current flow within the retina which leads to the generation of the *b*-wave should differ between vascularized mammalian species and other vertebrates.

5.2. Other ERG Potentials

The pattern of light-evoked changes in $[K^+]_o$ within the retina depends on many factors, including the adaptation state of the eye, the pattern of light stimulation, and the species involved. It is no surprise then, that, under a variety of conditions, many different components of the ERG are believed to be generated by K^+ current flow through Müller cells.

5.2.1. SLOW PIII RESPONSE

In the dark adapted retinas of rod dominated eyes, prolonged light stimulation generates a $[K^+]_o$ decrease in the rod inner segment layer (Oakley and Green, 1976; Karwoski and Proenza, 1978; Steinberg *et al.*, 1980). This $[K^+]_o$ decrease causes an efflux of K^+ from the distal end of Müller cells and a corresponding K^+ influx in the proximal portion of the cell. The resulting current flow within the retina generates a vitreal negative transretinal potential, the slow PIII response (Faber, 1969; Fujimoto and Tomita, 1979; Bolnick *et al.*, 1979; Newman and Odette, 1984).

5.2.2. INNER PLEXIFORM LAYER RESPONSES

Light stimulation often generates a prolonged $[K^+]_o$ increase in the inner plexiform layer (Karwoski and Proenza, 1977; Kline *et al.*, 1978). This increase leads to a K^+ influx and depolarization of Müller cells. The resulting current flow within the retina generates the negative intraretinal *M*-wave which has been recorded in the mudpuppy (Karwoski and Proenza, 1977), frog (Karwoski *et al.*, 1978) and cat (Sieving *et al.*, 1986a). Under dark-adapted conditions in cat a similar K^+ increase in the inner plexiform layer is thought to generate the scotopic threshold response (Sieving *et al.*, 1986b).

A brief light flash delivered to a dark adapted eye can result in a $[K^+]_o$ increase in the inner plexiform layer having latencies of up to 60 sec (Karwoski *et al.* 1988). The resulting K^+ currents within Müller cells generate the *e*-wave of the ERG (Newman and Lettvin, 1978; Karwoski *et al.*, 1988).

Sieving and Steinberg (1985, 1987) have shown that the pattern ERG is generated in the proximal retina and resembles, in some respects, the *M*-wave response. Their work suggests that the pattern ERG may arise, at least in part, from Müller cell K^+ currents generated by $[K^+]_o$ variations in the inner plexiform layer.

6. REGULATION OF BLOOD FLOW

Localized increases in neuronal activity in the brain (and presumably the retina) lead to dilation of CNS blood vessels and increases in regional blood flow (Roy and Sherrington, 1890; Fox and Raichle, 1984). Paulson and Newman (1987) have proposed that glial cell K^+ siphoning may be instrumental in coupling increased neuronal activity with increased blood flow. Arteries and aterioles dilate when exposed to increases in $[K^+]_o$ (Wahl, 1985). As discussed above, neuronal activity within the retina leads to a buildup of $[K^+]_o$, an influx of K^+ into Müller cells, and a K^+ efflux from regions of high K^+ conductance (endfeet and perhaps the processes surrounding blood vessels). The K^+ released by Müller cell processes terminating on retinal vessels may dilate these vessels and lead to increases in local blood flow. The same process may occur for astrocytes on the surface of the retina and for astrocytes within the brain (Paulson and Newman, 1987). It has also been suggested that Müller cells participate in retinal vasomotor control under conditions of hypercapnia and hypoxia (Tsacopoulos, 1977).

7. ASTROCYTE PHYSIOLOGY

A great deal is known about the electrophysiology of astrocytes in the CNS. Like Müller cells, they are almost exclusively permeable to K^+ (Ransom and Carlini, 1986). And, like Müller cells, their K^+ conductance is localized, to a great extent, in their endfeet (Newman, 1986b). They also possess a number of different voltage-dependent ion channels (Berwald-Netter *et al.*, 1986). The reader is referred to recent reviews of the subject (Ransom and Carlini, 1986; Berwald-Netter *et al.*, 1986).

Pflug and Nelson (1986) have recorded intracellularly from astrocytes on the surface of the cat retina. These cells have resting potentials of -60 to -70 mV and depolarize in response to visual stimulation. Their responses presumably are due to variations in $[K^+]_o$ generated by the activity of ganglion cells, with which they are intimately associated. Newman (unpublished observations) has recorded resting potentials of -85 to -95 mV from astrocytes on the surface of the rat retina.

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