

Regulation of Extracellular K^+ and pH by Polarized Ion Fluxes in Glial Cells: The Retinal Müller Cell

ERIC A. NEWMAN

Department of Physiology
University of Minnesota
Minneapolis, Minnesota

Müller cells, the principal glial cells of the retina, exhibit a high degree of functional and morphological polarization. An inward rectifying K^+ channel, the dominant ion channel in Müller cells, is localized preferentially to cell endfeet, which terminate on the vitreal surface of the retina and on blood vessels. Two acid/base transport systems, an Na^+/HCO_3^- cotransporter and a Cl^-/HCO_3^- anion exchanger, also are localized preferentially to the endfeet. These functional specializations facilitate the ability of Müller cells to regulate extracellular ion levels in the retina. Müller cells regulate extracellular K^+ levels by transporting K^+ away from the neural retina to the vitreous humor and the subretinal space. Müller cells may also regulate retinal CO_2 and pH by the combined action of cell carbonic anhydrase and acid/base transporters localized to the endfeet, and they may control blood flow by the depolarization-induced release of potassium and protons from cell endfeet onto blood vessels. The physiology of ion transport in CNS astrocytes is not understood as well as it is in Müller cells. The presence of inward rectifying K^+ channels and acid/base transporters in astrocytes, however, suggests that these cells may utilize mechanisms similar to those of Müller cells in regulating the extracellular microenvironment and in controlling blood flow. *The Neuroscientist* 2:109–117, 1996

KEY WORDS Müller cell, Glial cell, Polarized, K^+ channels, Na^+/HCO_3^- cotransport, Extracellular K^+ , Extracellular pH

Neuronal activity leads to substantial variations in the extracellular concentration of several ions within the CNS, including K^+ and H^+ (1, 2). These changes, in turn, can modulate subsequent neuronal behavior. Activity-induced increases in extracellular K^+ concentration ($[K^+]_o$) depolarize neurons and modulate neuronal excitability and synaptic transmission (3). Increases in extracellular H^+ concentration ($[H^+]_o$) block a variety of ion channels and ligand-gated receptors, leading to reduced neuronal excitability (4, 5). These effects can be substantial. In the retina, for instance, a decrease in extracellular pH (pH_o) of only 0.05 pH units reduces synaptic transmission between photoreceptors and second order neurons by 24% (4).

Glial cells play an essential role in muffing (6) activity-dependent changes in extracellular ion concentrations. Glial cells in the brain regulate $[K^+]_o$ in several ways. Potassium released from active neurons is removed from extracellular space by uptake into glial cells and by the transport of K^+ through glia to sites distant from the initial $[K^+]_o$ increase (1). Glial cells also possess several acid/base transport systems that can alter

pH_o . Recent evidence suggests that these cells generate, and perhaps regulate, $[H^+]_o$ variations (7, 8).

The case for glial cell regulation of extracellular ion levels is particularly strong in the retina, where a specialized glial cell, the Müller cell, has been extensively characterized. The accessibility of the retina to experimental manipulation and the ease of studying Müller cells in isolation have made the retinal glial cell preparation an ideal model system for characterizing the mechanisms by which glia regulate the neuronal microenvironment. This article will review the mechanisms by which Müller cells control extracellular K^+ and pH in the retina.

Retinal Müller Cells

Unlike other CNS regions, the retina is largely devoid of astrocytes and oligodendrocytes, the principal glial cells of the brain. Instead, the predominant glial cell is the Müller cell, a distinctive type of glia found exclusively in the retina. The Müller cell is a radial glial cell that spans the entire neural retina, from the inner limiting membrane at the vitreal border, to the photoreceptors in the distal retina.

The Müller cell has a distinctive polarized morphology that resembles the morphology of epithelial cells in many ways (Fig. 1). The distal end of the Müller cell contains fine microvillar extensions that project into the subretinal

E.A.N. is supported by National Institutes of Health grant EY 04077.

Address reprint requests to: Eric A. Newman, Ph.D., 6-255 Millard Hall, Department of Physiology, University of Minnesota, 435 Delaware Street S.E., Minneapolis, MN 55455.

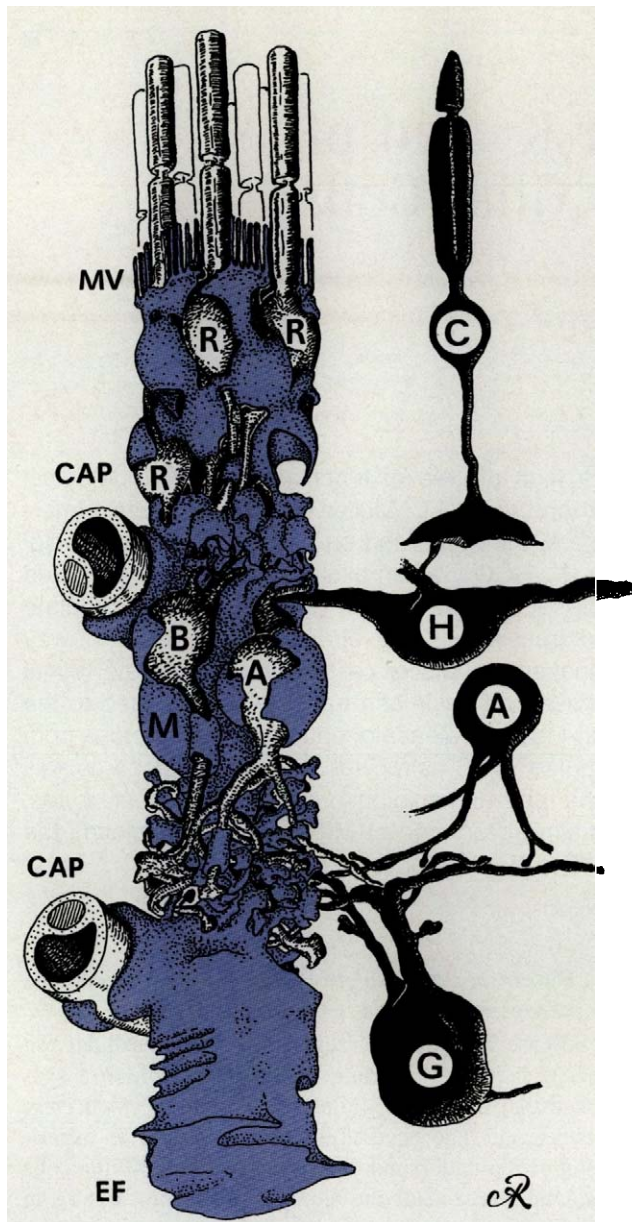


Fig. 1. Drawing of a Müller cell (*M*, shaded blue) of the vascularized mammalian retina with associated retinal neurons. An endfoot of the Müller cell (*EF*) terminates at the inner retinal border, adjacent to the vitreous humor. Additional endfeet terminate on capillaries (*CAP*). The photoreceptor end of the cell is covered with microvilli (*MV*), which project into the subretinal space surrounding photoreceptors. The Müller cell soma (*M*) lies in the inner nuclear layer. Neurons in the drawing include rod (*R*) and cone (*C*) photoreceptors; and horizontal (*H*), bipolar (*B*), amacrine (*A*), and ganglion (*G*) cells. Drawing courtesy of Andreas Reichenbach.

space surrounding photoreceptors. The proximal end of the cell forms the endfoot, a structure adjacent to a basal lamina (the inner limiting membrane) that separates the retina from the vitreous humor. Both the distal microvilli and the proximal endfoot resemble features typical of epithelial cells. The distal microvilli are analogous to the apical microvilli of epithelial cells, while the endfoot cor-

responds to the epithelial cell basal surface. Amphibian (but probably not mammalian) Müller cells are coupled by gap junctions, as are epithelial cells. Unlike epithelia, however, Müller cells are not linked by tight junctions and do not form a high resistance barrier.

The morphology of the Müller cell is more complex in mammalian species possessing vascularized retinas. In these species, the Müller cell processes additional endfeet bounded by basal laminae. These endfeet contact retinal blood vessels, both at the vitreal surface of the retina and within the inner nuclear layer. They are homologous to the endfeet of astrocytes that contact blood vessels in other regions of the CNS.

Müller cells are functionally as well as morphologically polarized, and have several types of ion channels and transport systems localized to specific cell regions. Potassium channels are localized preferentially to cell endfeet, as are certain acid/base transporters. Other surface molecules, including an Na^+/K^+ ATPase (9), a glutamate uptake carrier (10), lectin-binding glycoproteins (11), and cell adhesion glycoprotein CD44 (12), are also localized to specific areas on the Müller cell surface. In addition, orthogonal arrays of particles, assemblies of plasmalemmal particles characteristic of astrocytes and astrocyte-like cells (13), are strongly localized to cell endfeet terminating on the inner limiting membrane and on blood vessels (14).

In the following sections, we will consider how the functional polarization of the Müller cell facilitates the regulation of the retinal microenvironment. In particular, we will focus on the properties of K^+ channels and acid/base transport systems and the role they play in Müller cell regulation of $[\text{K}^+]_o$ and pH_o . Other reviews of Müller cells (1, 15) provide a comprehensive description of the properties and functions of these cells.

Inward Rectifying Potassium Channels

One physiological characteristic that distinguishes glial cells from neurons is their high conductance to K^+ . Although Müller cells possess a full complement of voltage-gated channels, including Ca^{2+} , Ca^{2+} -activated K^+ , A-type K^+ , and Na^+ channels (16, 17), most channels on the cell surface are inward rectifying K^+ channels (18-20). In Müller cells, as in other cells, these inward rectifying channels conduct best when the cell membrane potential is more negative than the K^+ equilibrium potential. Inward rectifying channels are responsible for the high conductance of Müller cells to K^+ at the resting membrane potential.

Channel Properties

The inward rectifying K^+ channel of amphibian and mammalian Müller cells has been characterized in whole-cell and single-channel voltage-clamp experiments. Its current-voltage relation is strongly rectifying (18-20). The channel has a conductance of ~ 30 pS in high $[\text{K}^+]_o$ and is blocked by micromolar concentrations of extracellular

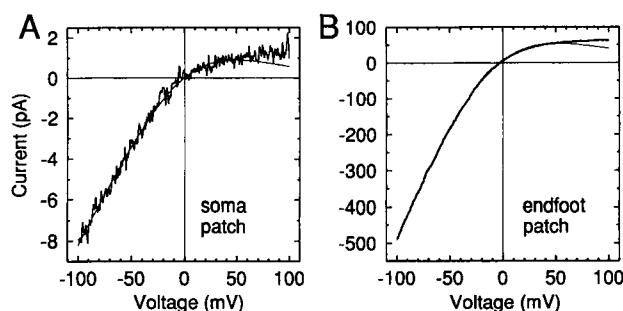


Fig. 2. Inward rectifying K^+ channels constitute the dominant conductance of Müller cells and, in amphibian species, are localized to the cell endfoot. These two multichannel cell-attached patch-clamp records from dissociated salamander cells illustrate that both soma and endfoot membranes have inward rectifying current-voltage properties. The density of inward rectifying channels is far greater on the endfoot, however (note the difference in the vertical scales). In this example, channel current is 62 times greater at the endfoot. (Reproduced with permission from *J Neurosci* 1993;13:3333–3345.)

Ba^{2+} (19, 20). The conductance of Müller cell inward rectifying channels follows the square root $[K^+]_o$ versus conductance relation seen in the inward rectifying channels of other systems (18, 19).

The inward rectifying conductance in Müller cells is modulated by several substances, including glutamate, dopamine, and thrombin, all of which reduce cell conductance (20, 21). These responses are mediated by several second messenger systems, including intracellular Ca^{2+} and cAMP.

Channel Localization

Müller cell inward rectifying channels are not distributed uniformly over the cell surface but are localized to specific cell regions. This striking feature is most prominent in amphibians, where ~90% of all Müller cell K^+ channels are localized at or near the basal membrane of the cell endfoot (22).

Channel localization has been studied using several techniques. Raising $[K^+]_o$ locally by focal ejections of K^+ onto the cell surface results in cell depolarizations proportional to the local value of membrane conductance. In amphibian cells, K^+ ejections onto the endfoot, and particularly onto the basal membrane of this structure, evoke much larger depolarizations than do ejections onto other cell regions (22, 23). Cell-attached patch-clamp recordings of inward rectifying channel currents produce similar results. Multichannel recordings from endfoot patches yield currents 38 times as large as currents recorded from the soma (Fig. 2) (19). Indeed, cell-attached recordings from the endfoot show that an extremely high density of channels is localized to the basal membrane. Multichannel currents as large as 890 pA (corresponding to 320 channels per patch) have been recorded (19).

The distribution of Müller cell K^+ channels in mammals is more complex. In cells from vascularized mam-

malian retinas, a high density of K^+ channels is found in several cell regions, including the endfoot, the soma, and, in the cat, the apical end of the cell (23). In the monkey, the density is highest near the soma region, where K^+ channels are probably localized to cell endfeet that terminate on blood vessels in the inner nuclear layer (23).

Regulation of Extracellular Potassium

Neuronal activity generates substantial increases in $[K^+]_o$. (1). In the brain, natural sensory stimuli increase the resting $[K^+]_o$ of ~3 mM by up to 1 mM (24). In the retina, light stimulation results in $[K^+]_o$ increases of up to 1 mM in the synaptic layers and decreases of 2 to 3 mM in the subretinal space surrounding the photoreceptors (which hyperpolarize when stimulated) (25, 26). Under intense electrical stimulation or pathological conditions, such as epileptic activity, $[K^+]_o$ in the brain can approach 12 mM (27).

Glial cells remove excess K^+ released into extracellular space by several mechanisms. Net K^+ influx into glia occurs by both passive (K^+ and Cl^- influx) and active (Na^+/K^+ ATPase) uptake processes (1). Glia also transfer K^+ from sites of neuronal release by a “spatial buffer” mechanism (28)—excess K^+ flows into glial cells and generates a cell depolarization that drives out an equal amount of K^+ from other cell regions. This K^+ current results in the transfer of K^+ from regions where $[K^+]_o$ is high to regions where $[K^+]_o$ is lower.

Müller cells remove excess K^+ from the neural retina by a specialized form of the spatial buffer mechanism termed “ K^+ siphoning” (29) (see Box 1). When $[K^+]_o$ rises within the retina because of light-evoked neural activity, excess K^+ enters Müller cells. This K^+ influx drives out an equal amount of K^+ from other regions of the cell. Potassium efflux will be greatest in regions with high K^+ conductance or regions adjacent to low $[K^+]_o$. In amphibian Müller cells, efflux occurs, in large part, from the cell endfoot, where most K^+ channels are localized (29, 30). The K^+ released from the endfoot diffuses into the adjacent vitreous humor, which functions as an efficient sink.

Both inward and outward K^+ siphoning currents pass through Müller cell inward rectifying channels. The voltage- and K^+ -dependent properties of this channel enhance the clearance of K^+ from the retina. When $[K^+]_o$ is raised, channel conductance increases (19, 20). Thus, cell membrane conductance and K^+ currents will be enhanced in regions where $[K^+]_o$ is elevated. Computer simulations indicate that the properties of inward rectifying channels, compared with those of ohmic channels, lead to a 23% enhancement of the K^+ siphoning clearance rate when $[K^+]_o$ rises from 2.5 to 3.5 mM (19). The enhancement is increased to 137% when $[K^+]_o$ rises from 2.5 to 12 mM.

Potassium siphoning is a key mechanism for regulating retinal $[K^+]_o$. Light stimulation results in a $[K^+]_o$ increase in the vitreous humor near the retinal surface, as measured using K^+ -selective microelectrodes (30,

Box 1: Regulation of Extracellular Potassium by Müller Cell Potassium Siphoning

Efflux of K^+ from active neurons generates increases in $[K^+]_o$ that must be muffled to maintain normal neuronal function. In the retina, most K^+ is released within the second synaptic layer, the inner plexiform layer. A substantial fraction of this K^+ is removed from extracellular space by a current flow through Müller cells. The excess K^+ enters Müller cells, depolarizes the cells, and drives out an equal amount of K^+ from other cell regions. In Müller cells of vascularized mammalian retinas (Fig. 3), K^+ efflux occurs from several regions with a high density of K^+ channels: 1) the endfoot at the inner border of the retina, where released K^+ diffuses into the vitreous humor; 2) the apical end of the cell, where K^+ diffuses into the subretinal space; and 3) the endfeet terminating on blood vessels. In the subretinal space, light stimulation evokes a $[K^+]_o$ decrease that increases the driving force for K^+ efflux from the apical microvilli of Müller cells. The release of K^+ onto blood vessels may participate in the control of retinal blood flow (Box 3). When $[K^+]_o$ in the retina is reduced to normal levels, K^+ is returned to neurons by a reversal of the K^+ current flow. The directed flow of K^+ current through high conductance regions of Müller cells is termed “ K^+ siphoning” (29). It is a specialized form of the “ K^+ spatial buffering” process (28).

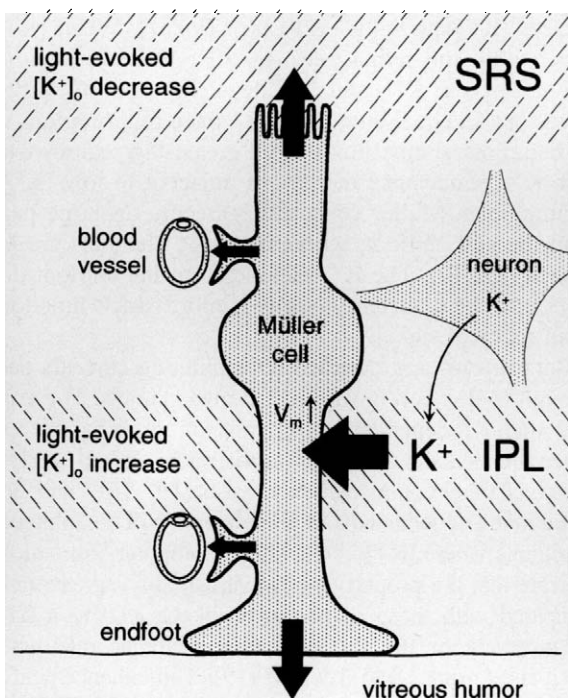


Fig. 3. Potassium siphoning in the mammalian retina. Heavy arrows, K^+ fluxes into and out of Müller cells; V_m , cell membrane potential; IPL, inner plexiform layer; SRS, subretinal space. The apical end of the Müller cell is at the top.

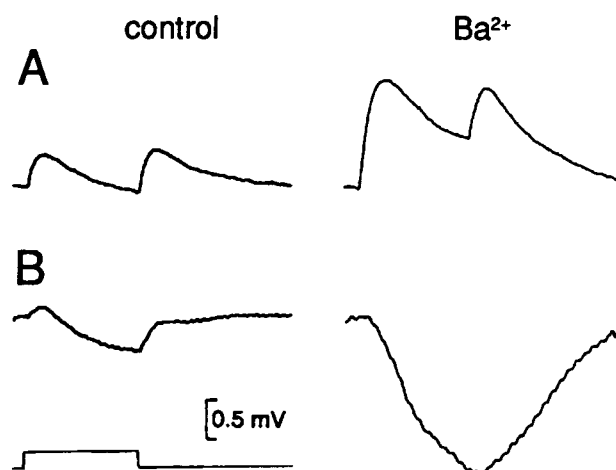


Fig. 4. Potassium siphoning through Müller cells plays an essential role in regulating $[K^+]_o$ in the retina. Light-evoked $[K^+]_o$ increases are recorded with a K^+ -selective microelectrode in the inner plexiform layer of the cat retina (A). When Müller cell K^+ channels are blocked by the addition of 3 mM Ba^{2+} , the light-evoked $[K^+]_o$ increase more than triples in amplitude. The light-evoked $[K^+]_o$ decrease in the subretinal space (B) more than quadruples when Ba^{2+} is added. The scale bar, 0.5 mV, represents ~ 0.1 mM $[K^+]_o$. (Modified and reproduced with permission from J Neurophysiol 1992;67:1201–1212.)

31). This vitreal $[K^+]_o$ increase is generated by a Müller cell K^+ siphoning current because the increase is nearly abolished when Müller cell K^+ channels are blocked by Ba^{2+} . Measurements of $[K^+]_o$ in the inner synaptic (plexiform) layer of the retina demonstrate the importance of this K^+ clearance mechanism. In amphibia, light-evoked $[K^+]_o$ increases more than double (31), and in cats, more than triple (32) when siphoning currents are blocked by Ba^{2+} (Fig. 4A).

In addition to the K^+ efflux from the endfoot, K^+ is also directed out of the apical end of Müller cells into the adjacent subretinal space. Potassium efflux from the apical end of Müller cells occurs for two reasons: 1) a light-evoked $[K^+]_o$ decrease in the subretinal space, generated by K^+ transport into photoreceptors (25, 32), increases the driving force for K^+ efflux in this region; and 2) in mammalian species, particularly in the cat, the apical end of Müller cells has a high K^+ conductance. Thus, during light stimulation, a fraction of the K^+ released from neurons is transferred by a K^+ current to the subretinal space (31, 32). This component of the K^+ flux not only reduces the $[K^+]_o$ increase within the neural retina but also minimizes the $[K^+]_o$ decrease in the space surrounding photoreceptors (Fig. 4B).

As mentioned above, K^+ conductance in Müller cells is modulated by several substances. This suggests that the efficiency of K^+ clearance from the retina may be influenced by its physiological state, as the levels of neurotransmitters, such as glutamate and dopamine, vary. Potassium clearance may also be altered under pathological conditions, such as when thrombin levels rise because of a breakdown of the blood-retina barrier (20).

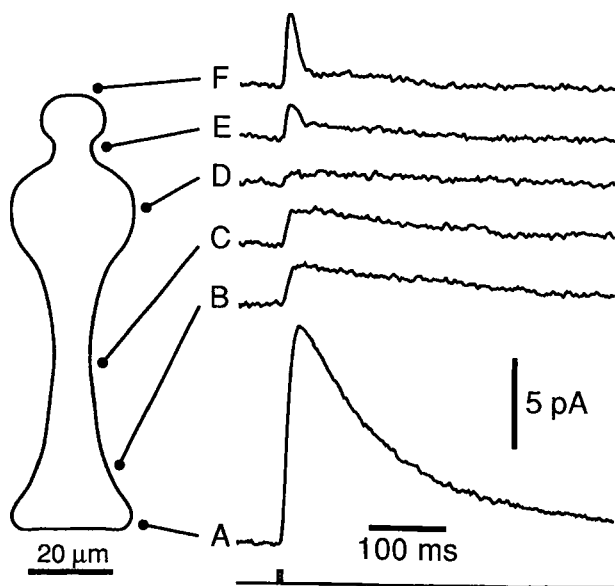


Fig. 5. Sodium/bicarbonate cotransport is localized to the cell endfoot in amphibian Müller cells. Focal increases in extracellular HCO_3^- , generated by HCO_3^- ejection (bottom trace), evoke influxes of Na^+ and HCO_3^- and generate the outward currents seen in these whole-cell voltage-clamp records. Ejection of HCO_3^- onto the endfoot (A) evokes the largest current, demonstrating that cotransporters are localized preferentially to this cell region. (Reproduced with permission from J Neurosci 1991; 11:3972–3983.)

Sodium/Bicarbonate Cotransport

Müller cells possess several acid/base transport systems, including an $\text{Na}^+/\text{HCO}_3^-$ cotransporter, a $\text{Cl}^-/\text{HCO}_3^-$ anion exchanger, and an Na^+/H^+ exchanger. These transport systems regulate intracellular pH in Müller cells and may contribute to changes in extracellular pH in the retina.

Sodium/Bicarbonate Cotransporter Properties

The Müller cell $\text{Na}^+/\text{HCO}_3^-$ cotransporter, like those of other cells, is electrogenic: a net negative charge is transferred as 3 HCO_3^- are transported along with each Na^+ (33, 34). Because the cotransporter is electrogenic, it is modulated by cell membrane potential. Cell depolarization favors an influx of Na^+ and HCO_3^- and results in an intracellular alkalization.

The Müller cell $\text{Na}^+/\text{HCO}_3^-$ cotransporter has been characterized in several ways. When isolated Müller cells are depolarized by raising $[\text{K}^+]_o$, intracellular pH, monitored by ratio imaging of the pH indicator dye, BCECF, becomes more alkaline (35, 36). The alkalization is HCO_3^- -dependent, Cl^- -independent, and is inhibited by DIDS, a cotransporter blocker. Cotransporter activity has also been monitored by recording the current generated when extracellular HCO_3^- is increased. Ejection of HCO_3^- onto Müller cells leads to an influx of Na^+ and HCO_3^- and to the generation of an outward current, as recorded in whole-cell voltage-clamp experiments (34) (Fig. 5).

Activation of the cotransporter generates an extracellular acidification as well as an intracellular alkalization. When local pH_o is monitored with a variant of the ratio imaging technique, a rapid acidification of the bathing solution surrounding dissociated cells is observed (35, 36) (Fig. 6).

Cotransporter Localization

Sodium/bicarbonate cotransporter sites, like K^+ channels, are localized preferentially to cell endfeet. The cotransporter has been localized in amphibian cells by monitoring currents evoked by local ejection of HCO_3^- onto the cell surface. The largest cotransporter currents are seen for HCO_3^- ejections onto the endfoot (34) (Fig. 5). The cotransporter has also been localized by monitoring the location of the extracellular acidification resulting from cotransporter activation. When the cotransporter is stimulated by raising $[\text{K}^+]_o$, extracellular acidification is greatest at the cell endfoot (35, 36) (Fig. 6).

Other Acid/Base Transport Systems

The Müller cell possesses a second HCO_3^- transport system, a $\text{Cl}^-/\text{HCO}_3^-$ anion exchanger. When Cl^- is removed from the extracellular bathing solution, an intracellular alkalization is observed. This alkalization, generated by an efflux of Cl^- and the associated influx of HCO_3^- , is HCO_3^- -dependent and is inhibited by DIDS, a blocker of the $\text{Cl}^-/\text{HCO}_3^-$ anion exchanger (35).

The $\text{Cl}^-/\text{HCO}_3^-$ exchanger also is localized preferentially to the cell endfoot. Immunohistochemical labeling indicates that the amphibian Müller cell possesses an anion exchanger expressed from the AE3 gene (37). Labeling is heaviest at the endfoot, indicating that anion exchange is localized to this cell region.

The Müller cell possesses the ubiquitous Na^+/H^+ exchanger that also plays an important role in regulating intracellular pH. The recovery of intracellular pH following a Müller cell acidification is dependent on the Na^+/H^+ exchanger. The recovery does not occur when Na^+ is removed from the extracellular solution and when amiloride, an Na^+/H^+ exchange blocker, is added (unpublished observations). It is not yet known whether the Na^+/H^+ exchanger is localized to specific regions of the Müller cell or is distributed uniformly over its surface.

Müller Cell Control of Extracellular pH

Neuronal activity results in a series of extracellular pH changes. In regions of the brain containing synapses, activity often generates a transient alkalization, followed by a prolonged acidification (2). The alkalization is generated by activation of both glutamatergic and GABAergic receptors (38) while the acidification is produced by acid and CO_2 efflux from active neurons (39). In the retina, a light-evoked extracellular alkalization dominates in both the photoreceptor and the inner plexiform (synaptic) layers (40, 41).

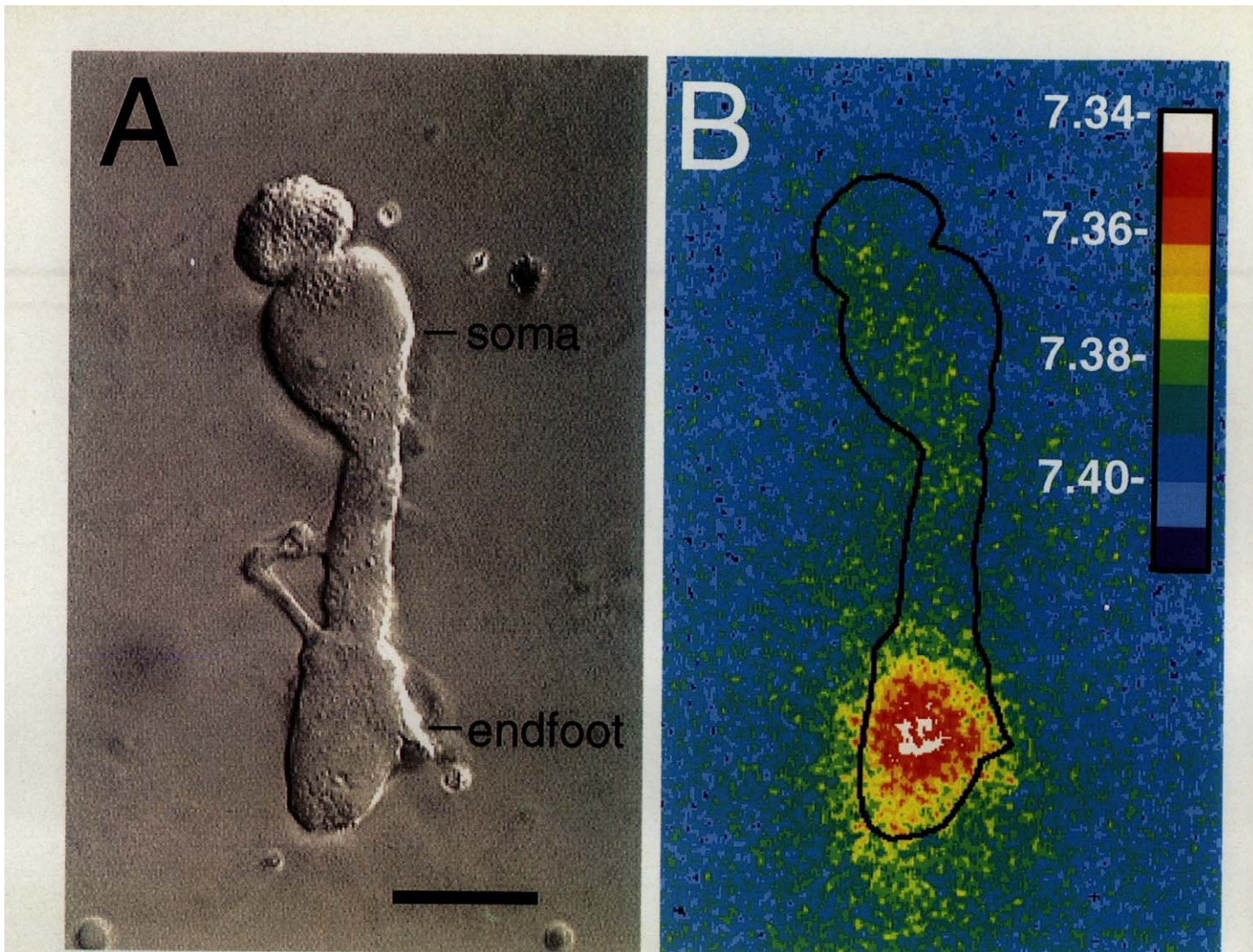


Fig. 6. The $\text{Na}^+/\text{HCO}_3^-$ cotransporter generates an extracellular acidification that is localized preferentially to the cell endfoot. Micrograph of a dissociated salamander Müller cell (A). Scale bar, 20 μm . Image of extracellular pH beneath the cell, as measured by imaging of a pH indicator dye fixed to the coverslip (B). The cell $\text{Na}^+/\text{HCO}_3^-$ cotransporter is activated by raising $[\text{K}^+]_o$. The resulting Na^+ and HCO_3^- influx into the cell generates an extracellular acidification, which is greatest at the endfoot. The pseudocolor image is calibrated in pH units (bar at right). (Reproduced with permission from J Neurosci 1996;16:159–168).

Glial cells contribute to activity-dependent pH variations in extracellular space. The glial cells in hippocampal slices generate an extracellular acidification when $[\text{K}^+]_o$ is raised (8). This acidification arises from stimulation of the electrogenic $\text{Na}^+/\text{HCO}_3^-$ cotransporter. As discussed above, the retinal Müller cells also generate a depolarization-induced extracellular acidification produced by activation of $\text{Na}^+/\text{HCO}_3^-$ cotransport (35, 36).

The glial acidification of extracellular space may serve an important regulatory function. The extracellular alkalization produced by synaptic activation can result in a substantial enhancement of synaptic potency (because of a release from H^+ block of presynaptic Ca^{2+} channels) (4). The acidification generated by glial cells will partially counter this alkalization and result in a stabilization of synaptic strength. In this way, activity-

dependent acid efflux from glial cells may be viewed as a component of a negative feedback system limiting neuronal excitability (42).

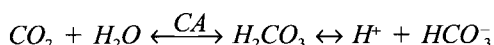
Müller cells may play such a pH regulatory role in the retina. Light-evoked neuronal activity depolarizes Müller cells and activates the $\text{Na}^+/\text{HCO}_3^-$ cotransporter. The resulting acid efflux from Müller cells will reduce the neuronally generated extracellular alkalization and stabilize overall retinal excitability. Although the acidification is greatest at the endfeet of Müller cells, acid efflux also occurs in other regions, including the synaptic layers.

Regulation of Retinal CO_2

The retina is one of the most metabolically active tissues in the body and produces substantial CO_2 , which must be removed efficiently to prevent tissue acidosis. Müller

Box 2: Regulation of CO₂ in the Retina

Active neurons generate CO₂, which must be removed efficiently to prevent acidosis. In the retina, photoreceptors are particularly active and produce abundant CO₂. Müller cells may aid in removing this CO₂ by converting it to HCO₃⁻ and H⁺. Although CO₂ has a high diffusion coefficient ($1.6 \cdot 10^{-5} \text{ cm}^2 \text{ s}^{-1}$) and, thus, can diffuse away from active neurons rapidly, its concentration in solution is only 1.3 mM (at pH 7.4). In contrast, although bicarbonate has a lower diffusion coefficient ($0.9 \cdot 10^{-5} \text{ cm}^2 \text{ s}^{-1}$), it is present at a concentration of 26 mM. Net HCO₃⁻ diffusional flux, thus, is 11-fold higher than that of CO₂ (45). Consequently, CO₂ can be removed from a region of neuronal activity more efficiently if it is first converted to HCO₃⁻. The conversion of CO₂ to HCO₃⁻ proceeds by the reaction that is catalyzed by the enzyme CA (43):



Müller cells, which contain both membrane-bound and cytoplasmic forms of CA (20, 44), may aid in CO₂ removal. The CO₂ released by photoreceptors is converted to HCO₃⁻ and H⁺, either within or at the surface of Müller cells. Bicarbonate and H⁺ (bound to mobile buffer molecules) then diffuse through the cells to the endfoot, where they are converted back to CO₂, and diffuse into the vitreous humor (Fig. 7, *path A*). This

“facilitated” CO₂ diffusion (45) may be augmented by the action of acid/base transporters at the endfoot. Sodium/bicarbonate cotransport and Na⁺/H⁺ exchange will transport HCO₃⁻ and H⁺ from the endfoot into the vitreous humor, thus reducing the concentration of these ions at the proximal ends of Müller cells and favoring further acid/base diffusion to the endfoot (Fig. 7, *path B*). This hypothetical CO₂ regulatory mechanism is termed “CO₂ siphoning” (44).

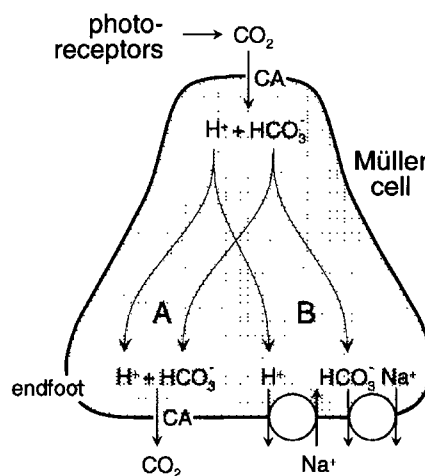


Fig. 7. Transport of CO₂ in Müller cells. CA, carbonic anhydrase.

cells may participate in this CO₂ homeostasis process. These cells, like other glia, possess high levels of carbonic anhydrase (CA), an enzyme that catalyzes the reversible hydration of CO₂ to H⁺ and HCO₃⁻ (43, 44). CA substantially increases the effective diffusion of CO₂ in solution by converting it to HCO₃⁻ (see Box 2). In the retina, the CA activity in Müller cells enhances the transfer of CO₂ from neural tissue to the vitreous humor.

The preferential localization of acid/base transport systems to Müller cell endfeet may augment this CO₂ transfer and lead to a polarized “CO₂ siphoning” transport mechanism (44) (Box 2). Following the hydration of CO₂ to H⁺ and HCO₃⁻, both ions will diffuse within Müller cells to the endfoot, where Na⁺/HCO₃⁻ cotransporters and, perhaps, Cl⁻/HCO₃⁻ exchangers will transport HCO₃⁻ into the vitreous humor. The activity of both transporters at the endfoot may establish an HCO₃⁻ gradient within the cell, favoring further diffusion to the endfoot and transport into the vitreous humor.

Control of Blood Flow

In both the brain and the retina, neuronal activity results in localized increases in blood flow (46, 47). This vascular response is the signal monitored when activity in

the intact brain is imaged with positron emission tomography and functional magnetic resonance imaging. The neuronal signals that modulate blood flow are not fully understood. Nitric oxide metabolism is involved but does not fully account for the vascular responses observed (48).

In addition to nitric oxide, both K⁺ and H⁺ modulate vascular smooth muscle tone. [K⁺]_o increases lead to arteriole dilation, as does a reduction in extracellular pH (49). The K⁺ effect arises from stimulation of smooth muscle Na⁺/K⁺ ATPase and the resulting cell hyperpolarization (50). The pH_o effect may arise from an H⁺ block of smooth muscle Ca²⁺ channels. Given the (approximately) fourth power relation between vascular diameter and hydraulic conductance (51), even a small increase in vessel diameter will lead to a significant increase in blood flow.

Glial cells may participate in the regulation of blood flow in the CNS (Box 3). The depolarization of glial cells modulates the extracellular milieu in two important ways: 1) depolarization generates a K⁺ efflux through K⁺ channels, resulting in a [K⁺]_o increase; and 2) depolarization generates an HCO₃⁻ influx through the electrogenic Na⁺/HCO₃⁻ cotransporter, resulting in an extracellular acid-

Box 3: Regulation of Blood Flow by Glial Cells

Neuronal activity leads to local increases in blood flow, delivering additional glucose and oxygen to active cells. Glial cells may partially mediate this vascular response. Glial cell depolarization resulting from neuronal activity leads to an efflux of K^+ through K^+ channels and to an influx of HCO_3^- through the electrogenic Na^+/HCO_3^- cotransporter (Fig. 8). The resulting rise in $[K^+]_o$ and decrease in pH_o may dilate blood vessels and lead to increased blood flow. In the retina, Müller cells are ideally suited to mediate vascular dilation. Both K^+ channels and cotransporter sites are localized preferentially to Müller cell endfeet, which terminate directly on blood vessels.

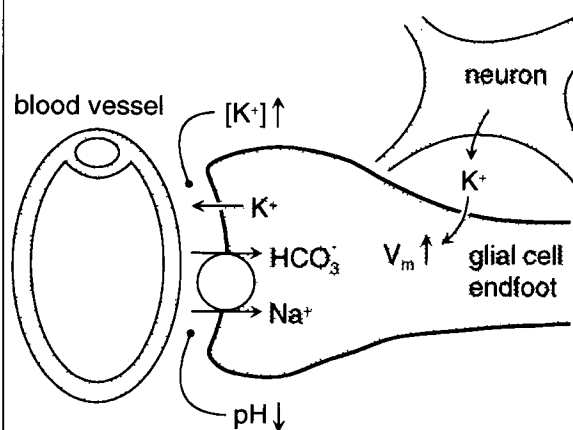


Fig. 8. Glial cell regulation of blood flow. V_m , cell membrane potential.

fication. Both K^+ channels and Na^+/HCO_3^- cotransporters are localized to the endfoot in Müller cells. Because many Müller cell endfeet terminate on blood vessels in the mammalian retina, activity-induced $[K^+]_o$ increases and extracellular acidifications will be large at vessel walls.

Müller cells, thus, are ideally suited to modulate blood flow in response to changes in neuronal activity. Elevated activity leads to an increase in $[K^+]_o$ and $[H^+]_o$ in the narrow space between cell endfeet and the smooth muscle cells of vessels. These increases may induce vessel dilation and lead to a local increase in blood flow. This signaling mechanism would have the further advantage of being extremely rapid. Communication between active neurons and the vessels would occur principally by the electrotonic spread of current through Müller cells, rather than by diffusion through intracellular or intercellular pathways.

Astrocytes

Do astrocytes in other regions of the CNS help to regulate extracellular ion levels as Müller cells do in the

retina? The evidence suggests that these cells may, indeed, help to regulate the neuronal microenvironment. Astrocytes possess a number of K^+ channels, including an inward rectifier channel (52), which help to remove excess K^+ from extracellular space. Potassium released by active neurons is taken up and temporarily sequestered in glia by both active and passive uptake processes (1, 53). In addition, under the appropriate conditions, K^+ spatial buffer currents can be induced in networks of glial cells coupled by gap junctions (54). These currents are thought to regulate activity-dependent $[K^+]_o$ variations in a manner similar to that observed in Müller cells (55).

CNS astrocytes also possess a number of acid/base transport systems, including an Na^+/HCO_3^- cotransporter, a Cl^-/HCO_3^- anion exchanger, and an Na^+/H^+ exchanger (2, 56). The Na^+/HCO_3^- cotransporter has been shown to generate extracellular acidification when astrocytes are depolarized (8). In the CNS of the leech, this glial Na^+/HCO_3^- cotransporter muffles a transient extracellular alkalization produced by neuronal activity (57).

Astrocytes in the brain may also regulate blood flow in response to neuronal activity. The presence of K^+ channels and Na^+/HCO_3^- cotransporters in astrocytes ensures that both K^+ and acid will be released from these cells when they are depolarized by neuronal activity. As discussed above, increases in $[K^+]_o$ and $[H^+]_o$ result in vascular dilation and in increased blood flow.

Little is known about the distribution of channels and transporters over the surface of brain astrocytes. Astrocytes are, of course, morphologically polarized. Astrocytes possess multiple endfeet, which terminate on brain vessels and the pial surface and contain high densities of plasmalemmal orthogonal arrays of particles (13). Astrocytes, like Müller cells, may be functionally polarized, as well. Indeed, in amphibian astrocytes isolated from the optic nerve, K^+ conductance is localized preferentially to cell endfeet (58). Given the functional and morphological similarities between astrocytes and Müller cells, it is quite possible that astrocytes in the brain employ mechanisms similar to those of Müller cells in regulating the extracellular microenvironment and in controlling blood flow.

Acknowledgment

I thank Janice I. Gepner and Kathleen R. Zahs for their helpful comments on the manuscript.

References

1. Newman EA. Glial cell regulation of extracellular potassium. In: Kettenmann H, Ransom BR, editors. *Neuroglial cells*. Oxford: Oxford University Press 1995.
2. Chesler M. The regulation and modulation of pH in the nervous system. *Prog Neurobiol* 1990;34:401-427.
3. Rausche G, Igelmund P, Heinemann U. Effects of changes in extracellular potassium, magnesium and calcium concentration on synaptic transmission in area CA1 and the dentate gyrus of rat hippocampal slices. *Pflugers Arch* 1990;415:588-593.
4. Barnes S, Merchant V, Mahmud F. Modulation of transmission gain by protons at the photoreceptor output synapse. *Proc Natl Acad Sci U S A* 1993;90:10081-10085.

5. Traynelis SF, Cull-Candy SG. Proton inhibition of N-methyl-D-aspartate receptors in cerebellar neurons. *Nature* 1990;345:347–350.
6. Thomas RC, Coles JA, Deitmer JW. Homeostatic muffling. *Nature* 1991;350:564.
7. Grichtchenko II, Chesler M. Depolarization-induced alkalinization of astrocytes in gliotic hippocampal slices. *Neuroscience* 1994;62:1071–1078.
8. Grichtchenko II, Chesler M. Depolarization-induced acid secretion in gliotic hippocampal slices. *Neuroscience* 1994;62:1057–1070.
9. Reichenbach A, Hagen E, Schippel K, Bruckner G, Reichelt W, Leibnitz L. Cytotopographical specialization of enzymatically isolated rabbit retinal Müller (glial) cells: structure, ultrastructure, and 3H-ouabain binding sites. *Z Mikrosk Anat Forsch* 1988;102:897–912.
10. Brew H, Attwell D. Electrogenic glutamate uptake is a major current carrier in the membrane of axolotl retinal glial cells. *Nature* 1987;327:707–709.
11. Reichenbach A, Dettmer D, Bruckner G, Birkenmeyer M, Birkenmeyer G. Morphological variability, lectin binding, and Na⁺, K⁺-ATPase activity of isolated Müller (glial) cells from the rabbit retina. *Neurosci Lett* 1985;55:29–34.
12. Chaitin MH, Wortham HS, Brun-Zinkernagel A-M. Immunocytochemical localization of CD44 in the mouse retina. *Exp Eye Res* 1994;58:359–366.
13. Landis DMD. Membrane structure in mammalian astrocytes: a review of freeze-fracture studies on adult, developing, reactive and cultured astrocytes. *J Exp Biol* 1981;95:35–48.
14. Wolburg H, Berg K. Distribution of orthogonal arrays of particles in the Müller cell membrane of the mouse retina. *Glia* 1988;1:246–252.
15. Newman EA. Müller cells and the retinal pigment epithelium. In: Albert DM, Jakobiec FA, editors. *The principles & practice of ophthalmology*, vol. 1: basic sciences. Philadelphia: WB Saunders 1994; 398–419.
16. Newman EA. Voltage-dependent calcium and potassium channels in retinal glial cells. *Nature* 1985;317:809–811.
17. Chao TI, Skachkov SN, Eberhardt W, Reichenbach A. Na⁺ channels of Müller (glial) cells isolated from retinæ of various mammalian species including man. *Glia* 1994;10:173–185.
18. Brew H, Gray PT, Mobbs P, Attwell D. Endfeet of retinal glial cells have higher densities of ion channels that mediate K⁺ buffering. *Nature* 1986;324:466–468.
19. Newman EA. Inward-rectifying potassium channels in retinal glial (Müller) cells. *J Neurosci* 1993;13:3333–3345.
20. Puro DG, Stuenkel EL. Thrombin-induced inhibition of potassium currents in human retinal glial (Müller) cells. *J Physiol (Lond)* 1995; 485:337–348.
21. Schwartz EA. L-glutamate conditionally modulates the K⁺ current of Müller glial cells. *Neuron* 1993;10:1141–1149.
22. Newman EA. Membrane physiology of retinal glial (Müller) cells. *J Neurosci* 1985;5:2225–2239.
23. Newman EA. Distribution of potassium conductance in mammalian Müller (glial) cells: a comparative study. *J Neurosci* 1987;7:2423–2432.
24. Kelly JP, Van Essen DC. Cell structure and function in the visual cortex of the cat. *J Physiol (Lond)* 1974;238:515–547.
25. Oakley BI, Green DG. Correlation of light-induced changes in retinal extracellular potassium concentration with c-wave of the electroretinogram. *J Neurophysiol* 1976;39:1117–1133.
26. Karwoski CJ, Newman EA, Shimazaki H, Proenza LM. Light-evoked increases in extracellular K⁺ in the plexiform layers of amphibian retinas. *J Gen Physiol* 1985;86:189–213.
27. Lux HD. The kinetics of extracellular potassium: relation to epileptogenesis. *Epilepsia* 1974;15:375–393.
28. Orkand RK, Nicholls JG, Kuffler SW. Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. *J Neurophysiol* 1966;29:788–806.
29. Newman EA, Frambach DA, Odette LL. Control of extracellular potassium levels by retinal glial cell K⁺ siphoning. *Science* 1984;225: 1174–1175.
30. Karwoski CJ, Lu H-K, Newman EA. Spatial buffering of light-evoked potassium increases by retinal Müller (glial) cells. *Science* 1989;244: 578–580.
31. Oakley BI, Katz BJ, Xu Z, Zheng J. Spatial buffering of extracellular potassium by Müller (glial) cells in the toad retina. *Exp Eye Res* 1992; 55:539–550.
32. Frishman LJ, Yamamoto F, Bogucka J, Steinberg RH. Light-evoked changes in [K⁺]_o in proximal portion of light-adapted cat retina. *J Neurophysiol* 1992;67:1201–1212.
33. Boron WF, Boulpaep EL. The electrogenic Na/HCO₃ cotransporter. *Kidney Int* 1989;36:392–402.
34. Newman EA. Sodium-bicarbonate cotransport in retinal Müller (glial) cells of the salamander. *J Neurosci* 1991;11:3972–3983.
35. Newman EA. Acid/base transport in salamander Müller cells. *J Brain Res* 1996;37.
36. Newman EA. Acid efflux from retinal glial cells generated by sodium-bicarbonate cotransport. *J Neurosci* 1996;16:159–168.
37. Kobayashi S, Morgans WC, Casey JR, Kopito RR. AE3 anion exchanger isoforms in the vertebrate retina: developmental regulation and differential expression in neurons and glia. *J Neurosci* 1994;14: 6266–6279.
38. Chen JCT, Chesler M. Modulation of extracellular pH by glutamate and GABA in rat hippocampal slices. *J Neurophysiol* 1992;67:29–36.
39. Voipio J, Kaila K. Interstitial PCO₂ and pH in rat hippocampal slices measured by means of a novel fast CO₂/H⁺-sensitive microelectrode based on a PVC-gelled membrane. *Pflügers Arch* 1993;423:193–201.
40. Borgula GA, Karwoski CJ, Steinberg RH. Light-evoked changes in extracellular pH in frog retina. *Vision Res* 1989;29:1069–1077.
41. Yamamoto F, Borgula GA, Steinberg RH. Effects of light and darkness on pH outside rod photoreceptors in the cat retina. *Exp Eye Res* 1992;54:685–697.
42. Ransom BR. Glial modulation of neural excitability mediated by extracellular pH: a hypothesis. *Prog Brain Res* 1992;94:37–46.
43. Maren TH. Carbonic anhydrase: chemistry, physiology, and inhibition. *Physiol Rev* 1967;47:597–781.
44. Newman EA. A physiological measure of carbonic anhydrase in Müller cells. *Glia* 1994;11:291–299.
45. Gros G, Moll W, Hoppe H, Gros H. Proton transfer by phosphate diffusion—a mechanism of facilitated CO₂ transfer. *J Gen Physiol* 1976;67:773–790.
46. Fox PT, Raichle ME. Stimulus rate dependence of regional cerebral blood flow in human striate cortex, demonstrated by positron emission tomography. *J Neurophysiol* 1984;51:1109–1120.
47. Riva CE, Harino S, Shonart RD, Petrig BL. Flicker evoked increase in optic nerve head blood flow in anesthetized cats. *Neurosci Lett* 1991;128:291–296.
48. Akgoren N, Fabricius M, Lauritzen M. Importance of nitric oxide for local increases of blood flow in rat cerebellar cortex during electrical stimulation. *Proc Natl Acad Sci U S A* 1994;91:5903–5907.
49. McCulloch J, Edvinsson L, Watt P. Comparison of the effects of potassium and pH on the caliber of cerebral veins and arteries. *Pflügers Arch* 1982;393:95–98.
50. Haddy FJ. Potassium effects on contraction in arterial smooth muscle mediated by Na⁺, K⁺-ATPase. *FASEB J* 1983;42:239–243.
51. Purves MJ. *The physiology of the cerebral circulation*. New York: Cambridge University Press 1972.
52. Barres BA, Chun LLY, Corey DP. Ion channels in vertebrate glia. *Annu Rev Neurosci* 1990;13:441–474.
53. Ballanyi K, Grafe P, ten Bruggencate G. Ion activities and potassium uptake mechanisms of glial cells in guinea-pig olfactory cortex slices. *J Physiol (Lond)* 1987;382:159–174.
54. Gardner-Medwin AR. Analysis of potassium dynamics in mammalian brain tissue. *J Physiol (Lond)* 1983;335:393–426.
55. Dietzel I, Heinemann U, Lux HD. Relations between slow extracellular potential changes, glial potassium buffering, and electrolyte and cellular volume changes during neuronal hyperactivity in cat brain. *Glia* 1989;2:25–44.
56. O'Connor ER, Sontheimer H, Ransom BR. Rat hippocampal astrocytes exhibit electrogenic sodium-bicarbonate cotransport. *J Neurophysiol* 1994;72:2580–2589.
57. Rose CR, Deitmer JW. Evidence that glial cells modulate extracellular pH transients induced by neuronal activity in the leech central nervous system. *J Physiol (Lond)* 1994;481:1–5.
58. Newman EA. High potassium conductance in astrocyte endfeet. *Science* 1986;233:453–454.