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Intracellular pH Transients of Mammalian Astrocytes

Mitchell Chesler[1,a] and Richard P. Kraig[1,2]

[1]Department of Neurology, Cornell University Medical College, New York, New York 10021, and [2]Department of Neurology and Committee on Neurobiology, The University of Chicago, Chicago, Illinois 60637

Intracellular pH (pH_i) is an important physiologic variable that both reflects and influences cell function. Glial cells are known to alter their functional state in response to a variety of stimuli and accordingly may be expected to display corresponding shifts in pH_i. We used fine-tipped, double-barreled, pH-sensitive microelectrodes to continuously monitor pH_i in glial cells *in vivo* from rat frontal cortex. Cells were identified as glia by a high membrane potential and lack of injury discharge or synaptic potentials. Continuous, stable recordings of pH_i from astrocytes were obtained for up to 80 min but typically lasted for approximately 10 min. Resting pH_i was 7.04 ± 0.02 with a membrane potential of 73 ± 0.9 mV (mean ± SEM; $n = 51$). With cortical stimulation, glia depolarized and became more alkaline by 0.05–0.40 pH ($n = 50$). During spreading depression (SD), glia shifted more alkaline by 0.11–0.78 pH ($n = 26$). After stimulation or SD, glia repolarized and pH_i became more acidic than at resting levels. Superfusion of the cortical surface with 0.5–2 mM Ba^{2+} caused glia to hyperpolarize during stimulation and completely abolished the intracellular alkaline response. The predominant pH response of the interstitial space during stimulation or SD was a slow acidification. With superfusion of Ba^{2+} an early stimulus-evoked interstital alkaline shift was revealed.

The mechanism of the intracellular alkaline shift is likely to involve active extrusion of acid. However, internal consumption of protons cannot be excluded. The sensitivity of the response to Ba^{2+} suggests that it is triggered by membrane depolarization. These results suggest that glial pH_i is normally modulated by the level of local neuronal activity.

In recent years, intracellular pH (pH_i) has been recognized as a modifiable parameter that both reflects and influences cell function (Busa and Nuccitelli, 1984). In the nervous system, activity-dependent shifts of intracellular ions, particularly Ca^{2+}, have received wide attention. Yet little is known about the corresponding behavior of pH_i. A few studies of nerve cells demonstrated an intracellular acid shift in response to depolarizing stimuli (Ahmed and Connor, 1980; Endres et al., 1986), although measurements of brain interstitial pH (pH_o) revealed both acid and alkaline transients (Urbanics et al., 1978; Kraig et al., 1983). While glial cells have been proposed to regulate pH_o (Swanson and Rosengren, 1962), the relationship between glial pH, pH_o, and neuronal activity is still obscure.

During cortical stimulation, increases in interstitial K^+ (K^+_o) have been correlated with extremely rapid ionic shifts across glial membranes (Ballanyi et al., 1987). With comparable elevations of K^+_o, the metabolic rate of glial cells increased markedly (Orkand et al., 1973). Corresponding studies of glial pH_i have only recently become possible in the mammalian CNS. We now report the direct, continuous recording of pH_i from rat cortical glia *in vivo*. Our measurements demonstrate that glial pH_i undergoes large, rapid, alkaline and acid shifts during electrical activity and spreading depression (SD). Portions of these results have appeared in preliminary form (Chesler and Kraig, 1987a, b).

Materials and Methods

Male Wistar rats (30 animals, 250–400 gm) were anesthetized with halothane (5% induction, 3% maintenance during surgery, and 0.5–1.0% during electrical recordings) and spontaneously, then mechanically ventilated with a 30% O_2–N_2 mixture. During recording periods, anesthetized animals were immobilized by a subcutaneous injection (0.2 ml) of curare. A tail artery was cannulated and a craniotomy was made over motor cortex (centered 2 mm rostral and 3 mm lateral to bregma). Brain pulsations were reduced by drainage of cerebrospinal fluid via a cisternal incision, bilateral pneumothoraces, and application of a superfusion cup to the exposed pial surface. The base of the superfusion cup acted as a pressure foot and consisted of a 3 mm diameter #60 mesh stainless steel grid insulated with plastic film. Warm (37°C) superfusate consisted of a physiological Ringer's solution containing (in mM): Na^+, 143.5; K^+, 3.0; Ca^{2+} 1.5; Mg^{2+}, 1.4; Cl^-, 115; HCO_3^-, 26.4; gluconate, 9.6; and glucose, 5.0, which when aerated with nominally 5% carbon dioxide and 95% oxygen had a pH of 7.30–7.40 (modified from Bretag, 1969). $BaCl_2$ (0.5–2 mM) was directly added to normal Ringer's solutions.

Arterial pH, oxygen tension (P_aO_2), and carbon dioxide tension (P_aCO_2) were monitored with a Corning 158 blood gas analyzer (Ciba Corning Diagnostics Corp., Medfield, MA). Blood gas variables were stabilized prior to brain recordings and monitored periodically throughout experiments. Blood glucose was measured with a Glucometer (Miles Laboratories, Naperville, IL).

Construction of pH-sensitive microelectrodes for intracellular recording has been described (Chesler and Kraig, 1987a). Briefly, one side of a double-barreled microelectrode was backfilled with 100% N,N-dimethyltrimethylsilylamine (Fluka), heated to 200–300°C, and then backfilled with proton-sensitive cocktail based on the ligand tridodecylamine (Ammann et al., 1981). PBS was injected immediately behind the proton-sensitive cocktail. Reference barrels contained 0.5 M KCl or 0.6 M K_2SO_4 and had resistances of 20–70 MΩ. For interstitial recordings, electrodes were made similarly and reference barrels contained 0.15 M NaCl. K^+-sensitive electrodes used a valinomycin-based cocktail

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Correspondence should be addressed to Richard P. Kraig, Department of Neurology and Committee on Neurobiology, The University of Chicago, 5841 South Maryland Ave., Chicago, IL 60637.

[a] Present address: Departments of Neurosurgery & Physiology & Biophysics, New York University Medical Center, 550 First Ave., New York, NY 10016.

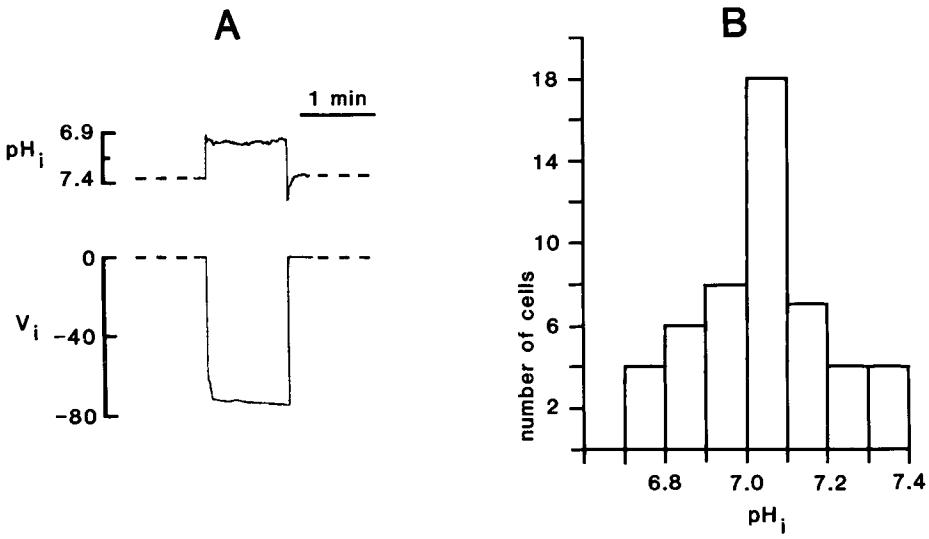

Figure 1. Resting pH_i of glial cells. *A*, Typical recording of pH_i from a neocortical glial cell. Upon penetration of a cell, pH recording (*top*) quickly settled to a new steady state while membrane potential (*bottom*) often took seconds to reach its maximum. Average membrane potential (51 cells) was 73 mV. *B*, Intraglial pH ranged from 6.73–7.38; average pH_i was 7.04. The most acidic cells were encountered in 3 animals. The most alkaline cells were invariably found at the pial surface in all animals.

(Fluka) and were calibrated in solutions of 1.5, 3, 6, 12, and 24 mM KCl in 0.15 M NaCl. K^+_o was referenced to the concentration of K^+ in the Ringer's. pH microelectrodes were calibrated in a series of phosphate buffers (pH 6.0–7.4) both before experiments and after recording from brain. pH_i recordings were referenced to superfusate pH that was routinely checked with a semimicro, glass pH electrode (410, Microelectrodes, Inc., Londonderry, NH).

Microelectrodes were connected to an Axoprobe A-1 amplifier system (Axon Instruments, Burlingame, CA). A 1 M KCl, 3% agar bridge placed on adjacent brain served as the distant indifferent electrode. Signals were filtered at 2 Hz, displayed on a strip chart recorder, and stored on videotape (DR-484; Neurodata Instruments, New York, NY). Electrical stimuli (0.2–2 msec duration, 40–100 volts) were delivered to the cortical surface via 0.5-mm-diameter, insulated, flattened stainless steel rods positioned 3.5 mm apart on opposite sides of the superfusion cup. All stimuli were delivered in trains (5–100 Hz, 840 msec duration) repeated at 1 Hz. Glial depolarizations less than 40 mV were achieved by stimulating the cortical surface at 5–50 Hz. To produce greater depolarizations, trains of stimuli were delivered at 100 Hz to evoke SD. During SD, a wave of elevated K^+_o propagates across the cortex, reaching levels in excess of 40 mM (Vyskocil et al., 1972).

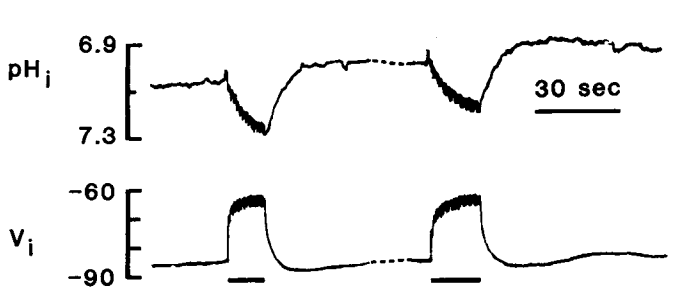

Figure 2. Response of glial cell to surface stimulation. Recording shown is from a glial cell at the pial surface with resting pH_i of 7.08 and membrane potential of 86 mV. During stimulation (20 Hz), membrane potential quickly fell to a new steady state of 62 mV, while pH_i shifted alkaline to 7.25. After stimulation, membrane potential hyperpolarized before returning to baseline. Glial pH shifted in the acid direction and then slowly began to return to baseline. A second 20 Hz stimulus was delivered 4 min after the first. Glial pH again showed a stereotypic alkaline–then acid response and reached an acidic plateau of 6.85. These results show that recurrent stimuli that are sufficiently close in time can progressively acidify glia.

Results

Resting pH_i of cortical glia

Cortical glia were identified on the basis of a high membrane potential (≥65 mV), lack of injury discharge, and absence of spontaneous action or synaptic potentials. In our preliminary study (Chesler and Kraig, 1987a) and in a previous detailed investigation (Takato and Goldring, 1979), injection of HRP into cortical cells with these electrical characteristics consistently labeled protoplasmic astrocytes. Glia were encountered throughout the cortex, but impalements occurred most regularly at or near the pial surface. These elements were presumably astrocytes in cortical layer I. Although neurons (judged by injury and spontaneous discharges) were also penetrated, membrane potentials were lower and cells were usually held for only seconds.

Following impalement of a glial cell, the pH_i record settled quickly to a steady state (Fig. 1*A*). Stable impalements were maintained for as long as 80 min, but typically lasted less than 10 min. In experiments on 19 animals, 51 cells with a membrane potential of 65 mV or greater (mean = 73 ± 0.9 mV, range 65–88 mV) had a resting pH_i of 7.04 ± 0.02 (range 6.73–7.38; see Fig. 1*B*). The corresponding arterial blood variables were: PO_2 123 ± 7 torr; PCO_2 38 ± 1 torr; pH 7.41 ± 0.01; glucose 155 ± 7 mM. All animals were normothermic (36.9 ± 0.1°C) and had a normal hematocrit (43 ± 0.5). Arterial blood pressure was mildly reduced (111 ± 5 mm Hg) from normal, presumably secondary to halothane.

Intracellular responses evoked by cortical stimulation

Stimulation of the cortical surface produced a rapid, sustained glial membrane depolarization, often followed by a prolonged hyperpolarization when stimulation ceased (Fig. 2). The depolarizing response ranged from 4–38 mV, depending on stimulus frequency and distance of the recording from the stimulating electrode. The glial depolarization can be attributed to an increase in K^+_o (Orkand et al., 1966), while the late hyperpolarizing response may result from a decrease of K^+_o and the accumulation of K^+ within the glial cell (for reviews, see Somjen, 1975; Walz and Hertz, 1983).

Membrane depolarization was always accompanied by an intracellular alkaline shift (Fig. 2). With prolonged stimulation,

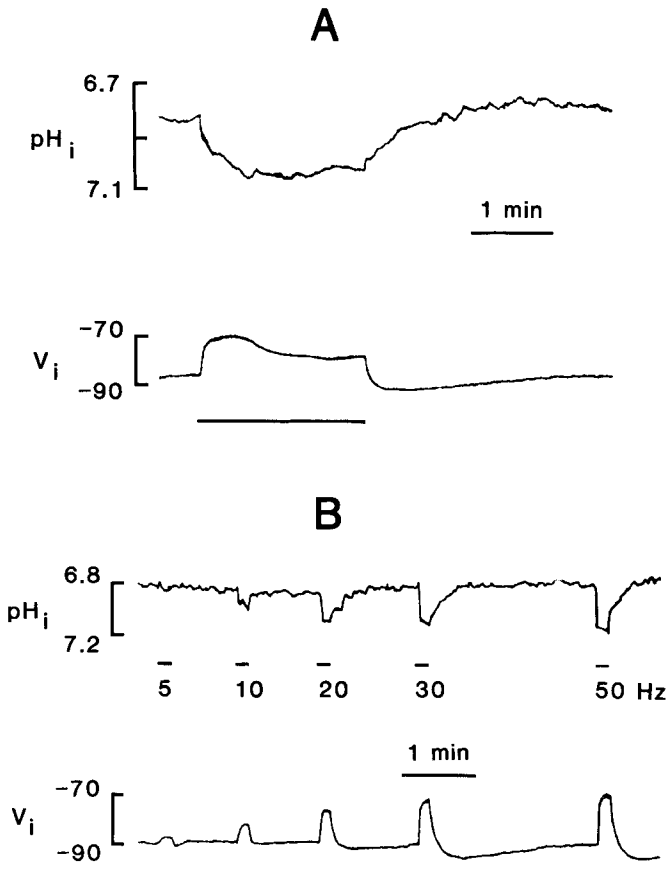

Figure 3. Influence of stimulus duration and frequency on glial alkaline shifts. Record shown is from a glial cell found less than 100 μm below the pial surface. *A,* During prolonged stimulation at 20 Hz (*bar*), pH_i shifted from 6.82 to a new steady state of 7.08. *B,* In the same cell, brief bursts of stimulation (*bars*) caused progressively larger depolarizations and alkaline shifts as stimulus frequency was increased.

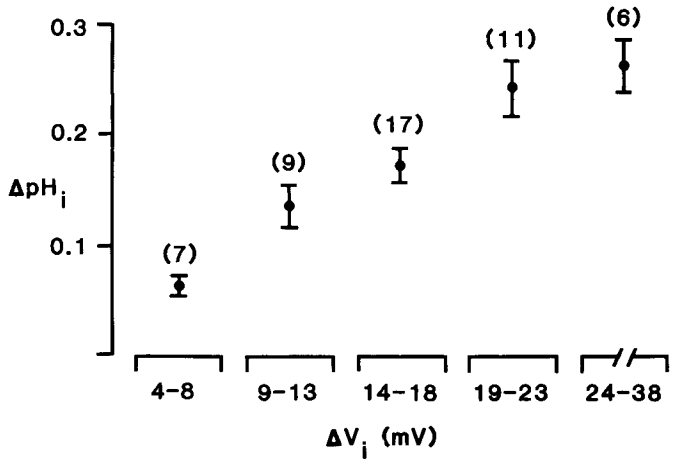

Figure 4. Relation of glial depolarization to pH_i. Depolarizations and alkalinizations were positively correlated ($r = 0.68$; 50 responses). Alkaline shifts were measured at the time of peak depolarization. Numbers in parentheses indicate number of responses for each range of depolarization.

pH_i either stabilized at an alkaline level (Fig. 3*A*) or slowly acidified (Chesler and Kraig, 1987a). After stimulation, pH_i recovered within tens of seconds and often continued into an acid rebound (Figs. 2, 3*A*).

Several lines of evidence indicated that these pH_i responses were genuine. First, when the stimulating electrode was raised from the cortical surface, there was neither a depolarization nor an alkaline shift recorded from glia. When the pH electrode recorded from interstitial space, only an acid shift was seen (see below). With poor penetrations the alkaline shift could still be evoked in glia despite membrane potentials of only 30–40 mV. However, in comparable neuronal penetrations, stimulation never produced an alkaline shift. Indeed, in nerve cells, only a slow acidification was observed (data not shown), in agreement with observations from frog spinal cord (Endres et al., 1986). Finally, the glial alkaline shift could be recorded only with functional pH microelectrodes. With age and use, pH electrodes became exceedingly slow owing to retraction of ion exchanger or plugging of the electrode tip. When glia were impaled with such electrodes, a normal membrane depolarization was observed, but there was little or no pH response. The alkaline shift therefore cannot be ascribed to a subtraction artifact.

A total of 50 stimulus-evoked alkaline shifts were recorded from 43 glial cells. For a given depolarization, the magnitude of the pH_i response varied considerably with impalements. This variability may reflect genuine differences among cells but could have been due, in part, to variability in the interstitial DC transient. (The latter assumption stems from the fact that a negative interstitial DC shift of 0–5 mV occurs with cortical stimulation. The recorded potential is the sum of this interstitial voltage and the transmembrane potential. Thus, intracellular voltages only approximate the transmembrane depolarization.) A nominal 15 mV depolarization was associated with a range of alkaline shifts from 0.11–0.28 (mean = 0.19 ± 0.02, $n = 9$). Raising the stimulus frequency or intensity, thereby further depolarizing a cell, generally increased the intracellular alkaline shift (Fig. 3*B*). When data from all cells were pooled, the correlation coefficient between the alkaline shift and intracellular depolarization was +0.68 (Fig. 4).

In 19 of 50 responses, the alkaline shift was followed by an acid rebound. The magnitude of the acid and alkaline shifts was not well correlated ($r = -0.25$). Factors governing the size of the acid shift were not studied systematically; however, the response appeared to increase with the duration of stimulation. Recovery from the acidification was slow. Thus, with repetitive periods of stimulation that were sufficiently close in time, cells became progressively more acidic (Fig. 2).

pH responses evoked by cortical SD

During repetitive cortical activity, K^+_o does not normally exceed a ceiling level of approximately 12 mM (Heinemann and Lux, 1977). Thus, the maximum depolarization achieved with stimulation was limited to roughly 40 mV. However, during SD, K^+_o exceeds this ceiling and can reach levels in excess of 40 mM (Vyskocil et al., 1972; Prince and Lux, 1973). Under these conditions, the above results would predict a large intracellular alkaline shift.

Glial pH_i transients during SD were analogous to those pH shifts evoked by electrical stimulation but were generally larger. An intracellular recording during SD is shown in Figure 5. SD was initiated by 2 brief trains of stimuli at 100 Hz. After a 15-sec delay, the membrane spontaneously depolarized as a wave of SD reached the recording site (note that the potential change recorded intracellularly represents the sum of the interstitial DC

Figure 5. Glial pH_i during SD. Record shown is from a glial cell found approximately 1000 μm below the pial surface. SD was initiated by 2 brief trains of 100 Hz surface electrical stimulation. Glial response to SD was similar in waveform to, but larger in magnitude than, that seen with cortical stimulation.

shift (Fig. 9) and the actual transmembrane potential). Concurrent with the intracellular depolarization, an alkaline shift of about 0.3 occurred. With repolarization, pH_i recovered and continued into an acid rebound as the membrane potential hyperpolarized. For 26 SDs, the peak alkaline shift was 0.32 ± 0.04 (range 0.11–0.78) and the final acid shift above baseline was 0.19 ± 0.02 (range 0.03–0.39). Furthermore, as with stimulation (Fig. 2), with successive SDs glia became progressively acidotic (Fig. 6).

Comparison of pH_i and pH_o responses

To study how pH_o transients corresponded to the glial responses, stimuli were repeated following withdrawal of pH electrodes from cells. Figure 7*A* shows data from one such experiment. Here, repetitive stimulation produced an intracellular alkaline shift followed by an SD with an associated alkaline–acid response. The pH electrode was then withdrawn from the cell, and after 10 min the cortex was again stimulated (Fig. 7*B*). The interstitial response consisted of a negative DC shift of a few millivolts and a prolonged acid shift. After a delay, SD again resulted, as indicated by a 30 mV negative DC potential shift. The corresponding pH_o response consisted of an acid shift interrupted early in its onset by a brief alkaline transient, as previously reported (Mutch and Hansen, 1984). During SD, the rise of the interstitial acidification occurred mainly during the plateau of the interstitial DC shift (see Fig. 8*B*). The intracellular alkaline shift also coincided with the depolarizing phase of SD (Figs. 5, 6, 7*A*). Thus, the intracellular alkaline shift of SD corresponded temporally with the interstitial *acid shift*.

The pH_o responses during stimulation and SD showed little variation within the cortex. Figure 8*A* shows the time course of pH_o responses at different cortical depths (20 Hz surface stimulation). While there was some variability in the latency and rate of rise of the acid shift, the response was similar throughout the cortex. Rarely, a small alkaline shift (≤0.02) preceded the acidification, as has been noted in other regions of the CNS (Urbanics, 1978; Kraig et al., 1983; Endres et al., 1985; Carlini and Ransom, 1986; Chesler and Chan, 1988). However, in most instances there was either a small acidification or no pH change during the first seconds of stimulation.

The voltage response of SD at different cortical depths and the accompanying changes in pH_o are shown in Figure 8*B*. The DC potential waveforms and the triphasic pattern of acid–alkaline–acid shifts were similar throughout the cortex, although occasionally a prolonged, more complex recovery phase was observed (see Fig. 12). Mutch and Hansen (1984) also noted triphasic pH_o transients during SD in rat neocortex, although in other regions of the CNS, a biphasic alkaline–acid response was observed (Lehmenkuhler et al., 1981; Kraig et al., 1983; Somjen, 1984).

Effect of Ba^{2+} on the intracellular alkaline shift

The correlation of the intracellular alkalinization and glial depolarization suggested that the alkaline shift might be blocked by suppressing the depolarizing response. This could not be achieved by passing hyperpolarizing current because the large currents required to polarize the low-resistance glial membrane could not be passed by pH electrode reference barrels. However, Ballanyi et al. (1987) found that Ba^{2+}, by blocking glial K^+ channels (Walz et al., 1984), causes glial cells to hyperpolarize in response to cortical stimulation, despite a normal rise in K^+_o. Such hyperpolarization was blocked by ouabain and was therefore ascribed to electrogenic Na–K pump activity. If glial depolarization is a necessary antecedent of the alkaline shift, the response should therefore be abolished in the presence of Ba^{2+}.

The first approach was to superfuse the brain with Ba^{2+}-Ringer's (0.5–2 mM), impale cells, and stimulate the cortical surface. Under these conditions, few cells could be penetrated, and those elements that were impaled had low membrane potentials. This may be attributed to the depolarization of glial cells by Ba^{2+} (Walz et al., 1984; Ballanyi et al., 1987). Seven cells had a mean membrane potential of 34 ± 1.6 mV and pH_i of 7.17 ± 0.04. These elements were most likely glial cells based on their location at or near the pial surface. Consistent with the behavior of glia, cortical stimulation in the presence of Ba^{2+} caused a striking hyperpolarization (Fig. 9) (Ballanyi et al., 1987). Note

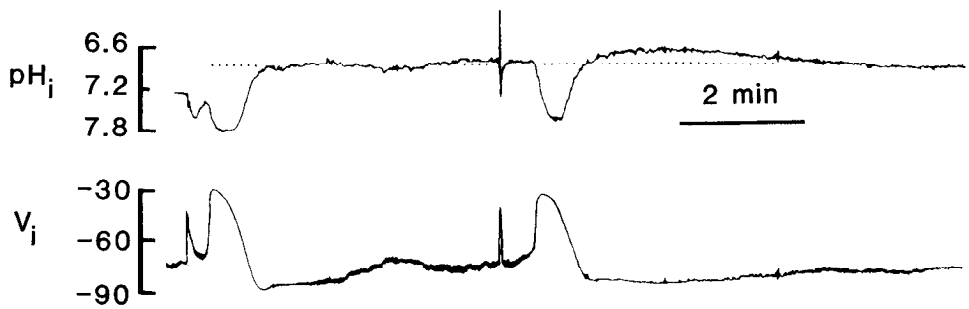

Figure 6. Glial pH_i change to successive SDs. Record shown is from a glial cell at the cortical surface. In this example, the initiating stimulus (100 Hz) briefly depolarized the cell and produced an alkaline shift. Before recovery of this transient, a wave of SD reached the recording site, giving rise to a large alkaline shift that approached a pH_i of 7.8. With recovery, a large, prolonged acid shift occurred. Three minutes later a second stimulus (100 Hz) was delivered, and after a 40-sec delay a second SD occurred. The net effect of these 2 SDs was to progressively acidify the cell to a pH of 6.6.

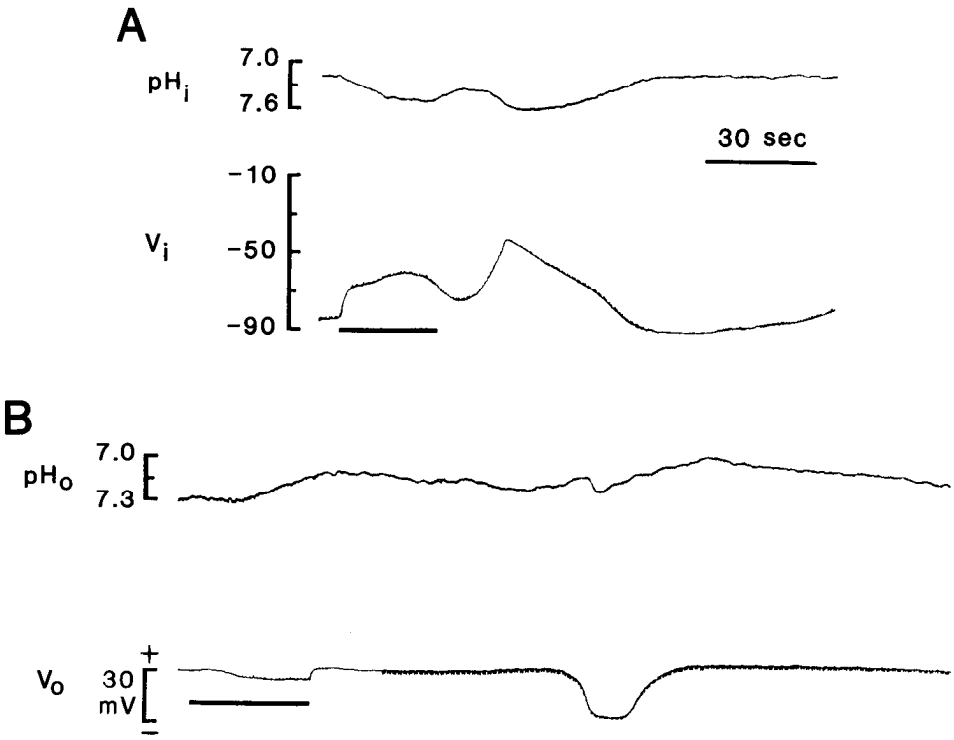

Figure 7. Comparison of intraglial and adjacent interstitial responses. *A*, Typical glial response to stimulation and SD. Repetitive stimulation of the cortical surface at 10 Hz (*bar*) caused the usual glial depolarization and alkaline shift. Shortly after the cessation of stimulation, SD occurred (seen as deflections in pH_i and V_i records after the stimulus bar). *B*, Next the electrode was withdrawn from the cell, and 10 min later a second 10 Hz stimulation (*bar*) was repeated. The predominant interstitial response to stimulation and SD was a slow acid-going transient.

that the ability of the cell to support a 30-mV hyperpolarization indicated that impalement-related membrane damage did not account for the low resting potential. There was no intracellular alkalinization in these experiments; the only pH_i response associated with the hyperpolarization was a small, slow, acid shift.

The effect of Ba^{2+} on superficial cells could be completely reversed, but required up to 1 hr of superfusion with normal Ringer's. When glial cells deeper than 500–1000 μm were impaled during superfusion of Ba^{2+}, membrane potentials were high (65–90 mV) and stimulation resulted in normal depolarizing responses and alkaline shifts. This indicated that effective penetration of Ba^{2+} was limited to the first several hundred micrometers of cortex.

A second experimental approach was to impale a superficial glial cell in normal superfusate, obtain a control response, superfuse with Ba^{2+}, and repeat the stimulus. In an *in vivo* preparation this protocol was technically difficult, but in 2 successful experiments results were similar. Figure 10 shows a control response consisting of an alkaline shift and depolarization, followed by an after-hyperpolarization. Superfusion with Ba^{2+} Ringer's (0.5 mM) depolarized the cell by about 10 mV. Stimulation in the presence of Ba^{2+} repeatedly evoked a hyperpolarizing response and a small intracellular acid shift.

To check whether the effects of Ba^{2+} were due to altered K^+_o responses, we studied K^+_o under identical conditions. Figure 11*A* shows that transition to Ba^{2+} Ringer's had no effect on the response of the K^+-selective microelectrode when placed in the superfusate above the brain. With the electrode lowered 200 μm into the cortex, transition to Ba^{2+} Ringer's produced a small, reversible decrease in baseline K^+_o. This slight fall in K^+_o caused by Ba^{2+} was noted in previous reports (Nicholson et al., 1976; Ballanyi et al., 1987). Figure 11*B* demonstrates that apart from

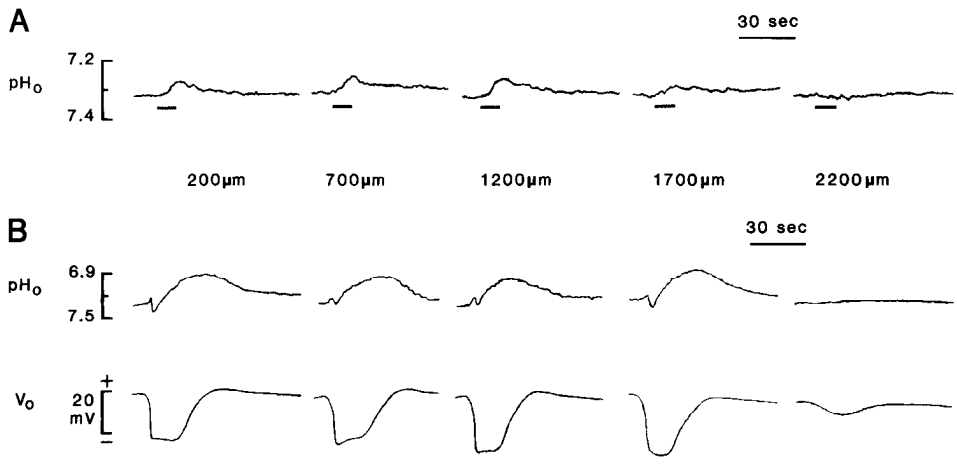

Figure 8. Interstitial depth profile of pH changes to stimulation and SD. *A*, response in depth to 20 Hz (*bar*) surface stimulation. *B*, Typical acid–alkaline–acid pH waveform of SD in the rat neocortex. Interstitial DC signals are shown to allow comparison to intracellular records (see text).

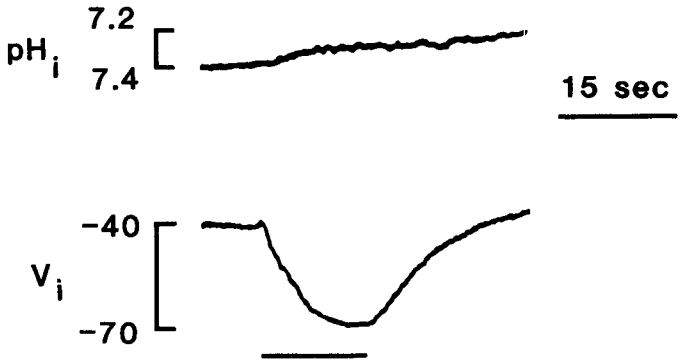

Figure 9. Response of glial cell to surface stimulation during superfusion with Ba^{2+}-Ringer's. Record is from a glial cell found less than 200 μm below the pia. The surface was exposed to 2 mM Ba^{2+}-Ringer's for approximately 20 min before the recording. Stimulation (19 Hz) caused a 30 mV hyperpolarization and a small acid-going shift.

this slight reduction in baseline K^+_o. Ba^{2+} did not significantly affect the stimulus-evoked rise of K^+_o.

Effect of Ba^{2+} on pH_o transients

Suppression of the intracellular alkaline shift by Ba^{2+} prompted study of its impact on pH_o transients. In the olfactory cortical slice, Ba^{2+} was reported to increase the early, stimulus-evoked alkaline transient (Endres et al., 1985). In rat cortex, this alkaline shift was rarely seen with stimulation (see above) but was consistently revealed by superfusion with Ba^{2+} Ringer's (Fig. 12). In addition, during SD, Ba^{2+} also enhanced the early alkaline transient (Fig. 12). These effects could be fully reversed, but again required as long as one hr of superfusion with normal Ringer's.

Discussion

The development of liquid membrane pH microelectrodes (Ammann et al., 1981) prompted several recent studies of brain pH_o. In these reports, the ultimate response to synchronous neuronal activity or SD was a prolonged acid shift (Kraig et al., 1983; Somjen, 1984; Nicholson et al., 1985; Siesjo et al., 1985; Carlini and Ransom, 1986). Intracellular measurements from nerve cells have revealed a simple acid shift in response to depolarizing stimuli (Ahmed and Connor, 1980; Endres et al., 1986), and in the present study the late response of glial cells was also a slow acidification (Figs. 2, 3*A*). These observations are consistent with metabolic studies, in which brain lactate and carbon dioxide production increased with activity (McIlwain and Bachelard, 1971). Thus, appearance of acidifying interstitial and intracellular responses in brain may be related to the generation of metabolic acids.

In the face of increased lactate and carbon dioxide production, an alkaline shift is seemingly paradoxical; nonetheless, marked alkaline responses can occur in separate brain compartments at the onset of stimulation or SD. Glial cells alkalinize (Figs. 2–7), while in the interstitial space both acid (Siesjo et al., 1985; this report) and alkaline (Urbanics et al., 1978; Kraig et al., 1983; Somjen, 1984; Nicholson et al., 1985; Carlini and Ransom, 1986; Chesler and Chan, 1988) shifts have been recorded. The relationship of the glial alkaline shift to these pH_o changes is unclear. In this study, an interstitial alkaline transient was noted only in the presence of Ba^{2+} (Fig. 12). This effect is similar to that noted in olfactory cortical slices in which Ba^{2+} enhanced an early interstitial alkaline shift. On the other hand, the glial alkaline shift was blocked by Ba^{2+}.

The disparate effects of Ba^{2+} on the interstitial and intracellular alkaline shifts indicates that the glial alkaline shift cannot give rise to interstitial alkalinization. Indeed, in this study the predominant interstitial response was an acidification that apparently masked an underlying alkalinizing process. The mechanism of the interstitial alkaline transient noted in other reports, and revealed by Ba^{2+} in this study, is unknown. It has been postulated that an extracellular alkaline shift is generated by acid influx into neurons through a channel-mediated pathway (Endres et al., 1986; Chesler and Chan, 1988). However, the occurrence of a glial alkaline shift and a predominant interstitial acidification suggests that a net efflux of acid may also occur from glial cells. Whether glial alkalinization may arise through a transmembrane pathway is considered below.

A *passive* efflux of acid from glial cells (or influx of base) can be excluded on thermodynamic grounds. At a pH_i and pH_o of 7.0 and 7.3, respectively, the equilibrium potential for H^+ or an acid–base equivalent (OH^-, HCO_3^-, NH_4^+, etc.) is −18 mV. Throughout cortical stimulation, the glial membrane potential remained far negative to this value. Thus, either some form of active acid transport across the plasma membrane or the internal consumption of protons is required to account for the intracellular alkaline shift.

Active transport or internal consumption of acid could be involved in several ways. Stimulation may simply result in enhancement of basal acid extrusion processes. Alternatively, with a constant rate of acid extrusion, an intracellular alkaline shift could result if an acid leak were diminished owing to either the closing of channels or the reduction of inward driving potential. Finally, the intracellular consumption of protons that occurs

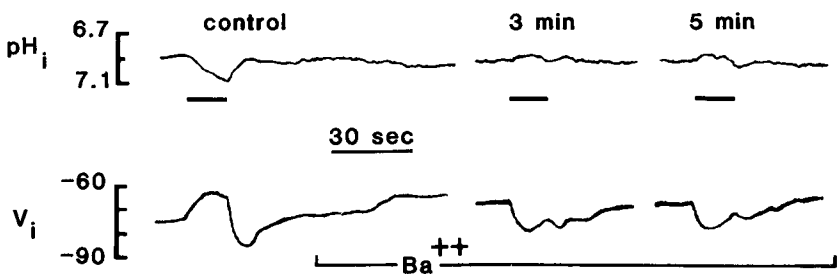

Figure 10. Serial changes in glial response to stimulation after exposure to Ba^{2+}. Records shown are from a cell approximately 100 μm below the pial surface. Control record at left shows the typical glial depolarization (*bottom*) and alkaline shift (*top*) during 10 Hz (*bar*) surface stimulation. With cessation of stimulation, the membrane potential hyperpolarized and then returned to baseline. Superfusion with 0.5 mM Ba^{2+}-Ringer's depolarized the cell by approximately 10 mV. Three and 5 min later 10 Hz stimulation (*bar*) caused a hyperpolarizing response with no alkaline shift.

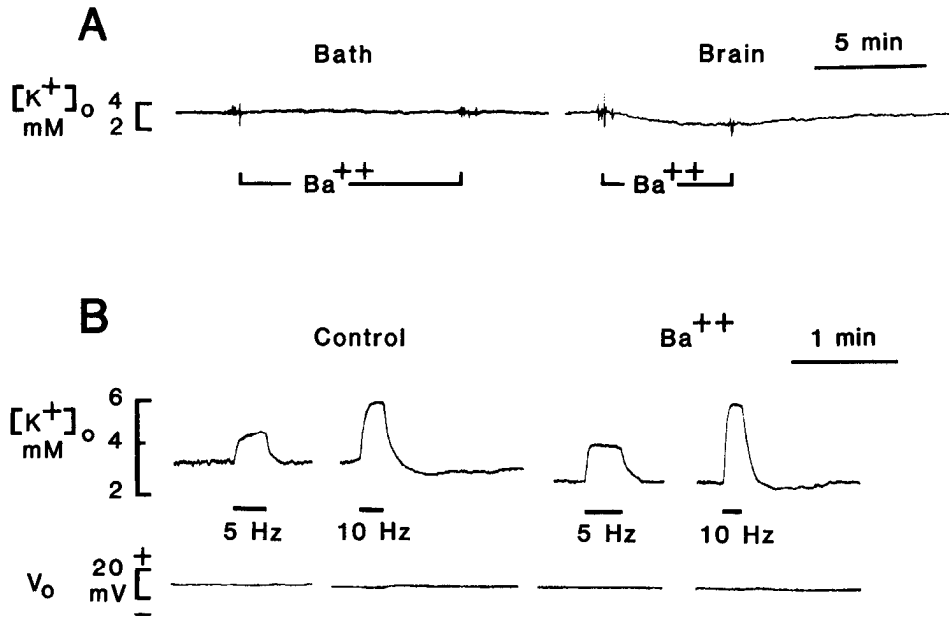

Figure 11. Influence of Ba^{2+} on K^+_o. *A*, Superfusion with 1 mM Ba^{2+} had no effect on electrode response in the bath but caused a small, reversible decrease of K^+_o in the brain (at 100 μm depth). *B*, Stimulus-evoked transients in K^+_o were not markedly altered by Ba^{2+}.

with hydrolysis of phosphocreatine (Siesjo, 1978) could be a basis for cytoplasmic alkalinization. Indeed, any cellular response that generates ATP faster than it is utilized would alkalinize cytoplasm (Busa and Nuccitelli, 1984).

Further insights may be gained by considering the addition of base (or removal of acid) required to generate a given alkaline shift. This can be estimated knowing the intracellular buffering power (β). β, in millimoles per liter, is defined as dB/dpH_i, where B is the concentration of added strong base (Roos and Boron, 1981). During an alkaline shift from pH 1 to pH 2, the total equivalent addition of strong base (ΔB) is given by

$$\Delta B = \int_{pH1}^{pH2} \beta \, dpH = \int_{pH1}^{pH2} [\beta_i + \beta_b] \, dpH \qquad (1)$$

where β_i is the non-HCO_3^- or "intrinsic" component of β and β_b is the CO_2/HCO_3^--dependent component (Roos and Boron, 1981). β_i may be attributed mainly to imidazole residues on proteins that have a pKa of 6.4–7.0 (Madias and Cohen, 1982) and may therefore not be markedly pH-dependent in the physiological range. As β_i is otherwise poorly defined, we treat this parameter as a constant of 20 mM (Roos and Boron, 1981). In contrast, β_b rises exponentially with pH_i and accounts for most of the intracellular buffering capacity. Ignoring the small contribution of CO_3^{2-}, β_b is given by $2.3[HCO_3^-]_i$ (Roos and Boron, 1981). Thus, solution of the Henderson–Hasselbach equation for $[HCO_3^-]_i$ and substitution into Equation (1) yields

$$\Delta B = (20 \text{ mM})(\Delta pH_i) + 2.3S'[PCO_2]e^{(-2.3pKa)} \cdot \int_{pH1}^{pH2} e^{(2.3pH_i)} \, dpH \qquad (2)$$

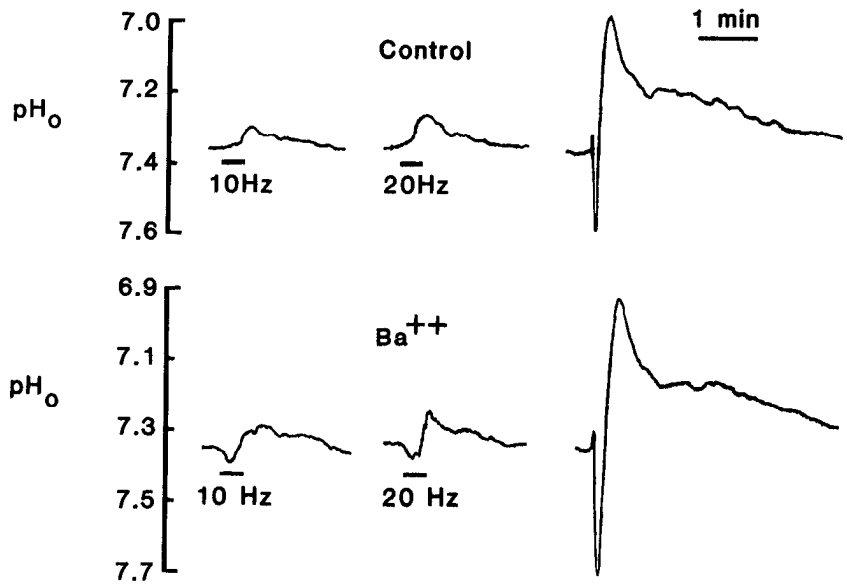

Figure 12. Influence of Ba^{2+} on pH_o responses to stimulation and SD. Superfusion with 1 mM Ba^{2+}-Ringer's (*bottom*) revealed an early alkaline response during 10 and 20 Hz stimulation (*bar*) and enhanced the early alkaline transient of SD (*right*).

where S′ is the solubility coefficient for carbon dioxide and PCO_2 is the partial pressure of carbon dioxide. Using an S′ of 0.0318 mM/torr, a pKa for carbonic acid of 6.13 (Siesjo, 1962), and a fixed PCO_2 of 46 torr (Ponten and Siesjo, 1966), integration of Equation (2) yields

$$\Delta B = (20 \text{ mM})(\Delta pH_i) + (1.1 \times 10^{-6} \text{ mM})(10^{pH2} - 10^{pH1}). \quad (3)$$

During cortical stimulation, a typical intraglial alkaline shift could range from pH 7.00 to 7.25. For this pH change, Equation (3) yields a ΔB of 14 mM. During the large alkaline shifts of SD, pH_i could increase from about 7.2 to 7.8 (Fig. 6). Such a change would require a ΔB of 64 mM.

This analysis indicates that the intracellular alkaline shift is generated by a substantial acid–base perturbation. To arise from a metabolic reaction, on the order of 10 mM substrate would need to be utilized within seconds. During SD, a far greater turnover would be required. In brain, phosphocreatine is found at a concentration of only 3–5 mmol/kg brain (Siesjo, 1978). It is therefore unlikely that its hydrolysis alone could account for the observed pH changes. Furthermore, an alkaline shift did not occur in the presence of Ba^{2+}, although utilization of phosphocreatine reserves was probable during the electrogenic hyperpolarization.

The magnitude of the underlying acid–base perturbation also places constraints on possible transmembrane mechanisms. If the alkaline shift resulted from enhanced acid transport or the reduction of an acid leak, the rate of acid extrusion would have to be greater than or equal to the rate at which ΔB increased. Since pH_i could rise by 0.2–0.3 within 10 sec (Fig. 2), acid extrusion would need to occur at about 50 mmol/liter/min. Based on studies of pH_i regulation, calculated acid extrusion rates for other mammalian cells (Aickin and Thomas, 1977; Simchowitz and Roos, 1985) are more than an order of magnitude lower. Nonetheless, the pH_i regulatory mechanisms of cortical glia have yet to be characterized. Furthermore, protoplasmic astrocytes, noted for their profuse elaboration of fine processes, have a high ratio of surface area to cytoplasmic volume. These cells may therefore be capable of effecting extremely rapid intracellular pH shifts via transmembrane fluxes.

Ballanyi et al. (1987) recently studied evoked K^+, Na^+, and Cl^- transients in glial cells of the olfactory cortical slice. In response to stimulation of the lateral olfactory tract, glia depolarized by 15–20 mV. In a matter of seconds, K^+_i and Cl^-_i increased by an average of 16.1 and 3.6 mM, respectively, while Na^+_i decreased by 6.0 mM. These data indicate that, on the average, intracellular electroneutrality would require an increase in unknown anions of about 7 mM. In the present study, a depolarization of 15 mV was associated with a mean intracellular alkaline shift of 0.19. Arising from a resting pH_i of 7.04, this shift would generate nearly 7 mM of intracellular HCO_3^-, thus accounting well for the unknown anion.

The results of Ballanyi et al. (1987), in concert with our own data, suggest that rapid acid–base shifts on the same order as the required ΔB can occur across glial membranes. A carrier or channel-mediated net acid efflux is therefore a plausible mechanism for the alkaline shift. This notion is consistent with the observed effects of Ba^{2+}. Thus, by preventing a net acid efflux, Ba^{2+} may diminish the rate of interstitial acid accumulation and thereby enhance the interstitial alkaline shift.

Ballanyi and colleagues further noted that in the presence of Ba^{2+}, stimulation produced a glial hyperpolarization, a fall in Cl^-_i, and a markedly reduced rise in K^+_i, despite a rise in K^+_o. Here, an intracellular anion deficit would not be expected, and, indeed, in the present study no glial alkaline shift (and hence no rise in intracellular HCO_3^-) occurred in the presence of Ba^{2+}. Thus, the glial alkaline shift does not depend on a rise in K^+_o per se. *In vitro* ion-substitution studies will be required to fully determine the ionic basis of the response.

Kelly and Van Essen (1974) demonstrated that glial cells in striate cortex depolarize 5–7 mV in response to simple visual stimuli. Our observations indicate that intracellular alkaline shifts up to 0.10 could accompany such activity. Thus, glial pH_i is probably modulated significantly with normal brain activity. Under pathological conditions, more pronounced changes would occur. During epileptic discharges, in which glia depolarize by as much as 20 mV (Sugaya et al., 1964), alkaline shifts of 0.20–0.30 can be expected. In the penumbra of focal ischemic loci, repetitive brain cell depolarizations can occur owing to prolonged spreading depressions (Nedergaard and Astrup, 1986). Here, the resultant intraglial acidosis could be an important factor influencing tissue viability (Kraig and Chesler, 1988).

Alkalinization might influence normal glial function in several ways. Both gap-junctional (Spray et al., 1981) and membrane K^+ conductance (Moody, 1984) can increase with a rise in pH_i and thereby enhance spatial buffering of K^+_o through the glial syncytium (Orkand et al., 1966). Glycolytic rate can rise markedly with intracellular alkalinization (Fidelman et al., 1982), allowing increased glucose utilization during neuronal activity. Finally, evidence that intracellular Ca^{2+} release (Siffert and Akkerman, 1987) and Ca^{2+}-dependent enzymes (Tkachuk and Men'shikov, 1981) may be highly pH dependent raises the intriguing notion that appropriately timed pH_i transients could have lasting structural and functional consequences for glia.

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