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MEMBRANE POTENTIAL DEPENDENCE OF INTRACELLULAR pH REGULATION BY IDENTIFIED GLIAL CELLS IN THE LEECH CENTRAL NERVOUS SYSTEM

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SUMMARY

1. We have measured the intracellular pH (pH_i) and membrane potential of identified glial cells in the central nervous system of the leech, *Hirudo medicinalis*, using double-barrelled pH-sensitive microelectrodes.

2. When extracellular K^+ concentration was increased, the glial membrane potential decreased and pH_i increased; lowering the extracellular K^+ concentration hyperpolarized the glial membrane and decreased pH_i. These pH_i changes were largely dependent upon the presence of CO_2–HCO_3^-; in nominally CO_2–HCO_3^--free saline solution, they were 50–80% smaller.

3. The steady-state pH_i of the glial cells in CO_2–HCO_3^--buffered saline solution strongly correlated with the membrane potential between −40 and −90 mV. The slope of this relationship was 60 mV/pH unit.

4. The neurotransmitter 5-hydroxytryptamine (50 μM), which hyperpolarizes the glial membrane, also produced a large, CO_2–HCO_3^--dependent decrease in pH_i. The size of the pH_i change depended upon the amplitude of the membrane hyperpolarization.

5. The increase in pH_i produced by the membrane depolarization in 20 mM-K^+ was abolished in Na^+-free saline. Removal of external Na^+ in the presence of 20 mM-K^+ reversed the pH_i increase.

6. The pH_i increase in 20 mM-K^+ was also inhibited by the stilbene 4,4-diisothiocyanostilbene-2′-disulphonic acid (DIDS, 0·5 mM). In a DIDS-poisoned preparation a small decrease of pH_i was observed in 20 mM-K^+ both in the presence and nominal absence of CO_2–HCO_3^-.

7. In neurones, neither CO_2–HCO_3^- nor 20 mM-K^+ produced an intracellular alkanization. The steady-state pH_i of several identified neurones was not correlated with the membrane potential.

8. We conclude that in glial cells, but not in neurones, the pH_i is dependent upon the membrane potential. This membrane potential dependence is due to the activity of the electrogenic Na^+–HCO_3^- co-transporter in the glial cell membrane.

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INTRODUCTION

In recent years glial cells have been shown to be functionally important partners of neurones in nervous systems, and are believed to play a crucial role for maintaining ionic homeostasis in the brain (Kuffler, 1967; Nicholson, 1980; Orkand, Coles & Tsacopoulos, 1983). In identified glial cells of the leech, active regulation of Na^+, K^+ and pH (Schlue & Wuttke, 1983; Deitmer & Schlue, 1987, 1989) has been reported. In rat astrocytes and in cultured mouse oligodendrocytes the intracellular pH has also been shown to be actively maintained alkaline with respect to the electrochemical H^+ equilibrium (Chesler & Kraig, 1987; Kettenmann & Schlue, 1988).

The three main mechanisms of intracellular pH regulation in glial cells are Na^+–H^+ exchange, Cl^-–HCO_3^- exchange, and Na^+–HCO_3^- co-transport (Kimelberg, Biddlecome & Bourke, 1979; Bourke, Kimelberg & Daze, 1987; Deitmer & Schlue, 1987, 1989; Kettenmann & Schlue, 1988; Astion & Orkand, 1989). The last, Na^+–HCO_3^- co-transport, operates with a proposed stoichiometry of 1:2, i.e. two HCO_3^- ions are carried with one Na^+ ion into the glial cells (Deitmer & Schlue, 1989), resulting, therefore, in a net outward current whenever this co-transporter is stimulated. The activated electrogenic Na^+–HCO_3^- co-transporter thus tends to hyperpolarize the membrane. This hyperpolarization appeared to be larger at more positive membrane potentials (Deitmer & Schlue, 1989). We were interested to see how the membrane potential affects the intracellular pH of these glial cells and in particular to what degree Na^+–HCO_3^- co-transport mediates any of these intracellular pH changes. We therefore measured the intracellular pH and the membrane potential of neuropile glial cells of the leech central nervous system and varied the membrane potential by altering the extracellular K^+ concentration. Our results suggest that, due to the voltage dependence of electrogenic Na^+–HCO_3^- co-transport, the intracellular pH is strongly correlated with the membrane potential in these glial cells. A preliminary report has been communicated to the Physiological Society (Deitmer & Szatkowski, 1989).

METHODS

The experiments were performed on identified glial cells and the Retzius neurones of the central nervous system of the leech *Hirudo medicinalis*. The preparation and dissection procedures used to isolate single ganglia have been described before (Deitmer & Schlue, 1981; Schlue & Deitmer, 1984). The isolated ganglia were pinned by their connectives and lateral nerve roots, ventral side upwards, to the silicone rubber base of a small experimental chamber with a volume of less than 0·2 ml. They were superfused with leech saline at a rate of 15–20 bath volumes/min. The experiments were done on the anterior and the posterior neuropile glial cells, and some on identified neurones including the Retzius cells and the lateral and medial noxious and pressure cells. The selection and identification of the glial and nerve cells followed criteria described previously (Deitmer & Schlue, 1981, 1987). All experiments were done at room temperature (22–25 °C). The numbers given are means ± S.D. of the mean.

Physiological solutions

The normal leech saline (nominally HCO_3^- free) had the following composition (in mM): NaCl, 105; KCl, 4; $CaCl_2$, 1·8; $MgCl_2$, 5; HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid) adjusted to pH 7·40 (±0·02) with NaOH, 10. This solution contained approximately

155 μM-HCO_3^-, due to the equilibration of air CO_2 (0·0314% or 8·4 μM) in the saline solutions, as calculated from the Henderson–Hasselbalch equation. Solutions buffered with CO_2–HCO_3^- instead of HEPES were equilibrated and continuously bubbled with nominally 2% CO_2–98% O_2 and contained 11 mM-$NaHCO_3$, pH 7·40. When the K^+ concentration was altered, equivalent amounts of NaCl were replaced with KCl. Na^+-free solutions were made by replacing Na^+ with *N*-methyl-D-glucamine (120 mM) neutralized with HCl and/or CO_2 as appropriate. In some experiments the stilbene DIDS (4,4-diisothiocyanostilbene-2′-disulphonic acid, 0·5 mM), 5-HT (5-hydroxytryptamine, 50 μM) or carbachol (0·1 mM) was applied directly to the saline shortly before use.

Microelectrodes

Theta-glass or 'side-by-side' double-barrelled borosilicate microelectrodes were used for recording pH and the reference (membrane) potential. The former, made using a two-stage pull procedure, were silanized for 2–3 min with pure hexamethyldisilazane (Serva) vapour at 40 °C, then baked at 100 °C. The latter were constructed, and both tested and calibrated, as described before (Schlue & Thomas, 1985; Deitmer & Schlue, 1987). The differential recording techniques were the same as in previous studies (Deitmer & Schlue, 1987, 1989).

RESULTS

The pH_i of leech neuropile glial cells is strongly dependent upon the buffer system used in the external solution; in a saline buffered with HEPES, the pH_i was $6·85 \pm 0·06$ ($\pm$S.D., $n = 25$), while in a saline solution buffered with 2% CO_2–11 mM-HCO_3^- the pH_i was $7·18 \pm 0·13$ ($n = 25$; see also Deitmer & Schlue, 1987, 1989). This alkalinization upon a change of the buffer is accompanied by a marked membrane hyperpolarization (Fig. 1). The changes in pH_i and membrane potential are due to the activation of inwardly directed, electrogenic Na^+–HCO_3^- co-transport (Deitmer & Schlue, 1987, 1989).

The effect of different K^+ concentrations

To depolarize the membrane the external K^+ concentration was increased from 4 to 20 mM. In the experiment shown in Fig. 1 the membrane potential decreased from -80 to -50 mV, and the pH_i increased from 7·1 to 7·45. After reduction of the external K^+ concentration to 4 mM again, membrane potential and pH_i returned towards their original values. This intracellular alkalinization in the presence of a raised external K^+ concentration is presumably due to stimulation of Na^+–HCO_3^- co-transport. Depolarization would bring the membrane potential further away from the reversal potential of the Na^+–HCO_3^- co-transporter, which was calculated to be -90 mV in normal steady-state conditions, assuming a stoichiometry of 1:2 (Deitmer & Schlue, 1989), thereby increasing the electrochemical gradient for the inwardly directed transport of HCO_3^-. We have tested this hypothesis in the following experiments.

Changes in pH_i at different external K^+ concentrations between 1 and 20 mM were measured in the presence (Fig. 2*A*) and in the nominal absence (Fig. 2*B*) of CO_2–HCO_3^-. In the presence of CO_2–HCO_3^- large, reversible changes of pH_i were measured in different external K^+ concentrations. Increasing the external K^+ concentration to 10 and 20 mM depolarized the membrane and produced a prominent intracellular alkalinization. Lowering the external K^+ concentration to 1 mM, which hyperpolarized the membrane, produced a marked decrease of pH_i. In the nominal absence of CO_2–HCO_3^- the absolute changes in pH_i were much smaller, and the rate

of the pH_i changes was considerably reduced. In the experiments shown, when increasing the external K^+ concentration from 4 to 20 mM, the maximum rate of intracellular alkalinization was 0·06 pH units/min in the presence of CO_2–HCO_3^-, and 0·024 pH units/min in the nominal absence of CO_2–HCO_3^- (where the HCO_3^- concentration is approximately 155 μM, see Methods). Similar values were obtained in two further experiments of this kind.

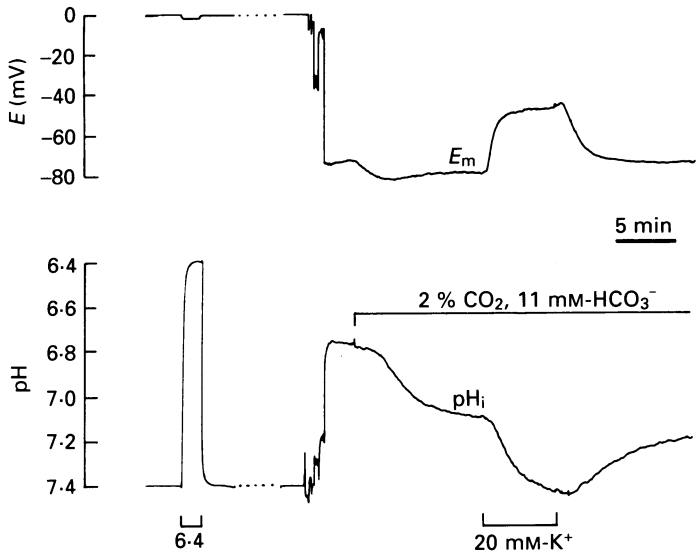

Fig. 1. Calibration of a double-barrelled pH-sensitive microelectrode and subsequent impalement into a neuropile glial cell. The reference and membrane potential, E, and E_m (upper trace), and the extracellular and intracellular pH, pH_i (lower trace), respectively, are recorded. Exchange of the HEPES-buffered saline by a CO_2–HCO_3^--buffered saline and increasing the external K^+ concentration affect both pH_i and E_m.

The pH_i and the membrane potential

From steady-state measurements of the pH_i in forty-five different neuropile glial cells in CO_2–HCO_3^--buffered saline there appeared to be a strong dependence of the pH_i on the membrane potential (Fig. 3). Extending the membrane potential range with experiments at different external K^+ concentrations confirmed this correlation. At a membrane potential of −45 mV, pH_i was around 7·5, while at a membrane potential of e.g. −80 mV, the pH_i was near 7·0. There appeared to be a linear relationship between membrane potential and pH_i between potential values of −40 and −90 mV. The dependence of the steady-state pH_i and membrane potential was strongly correlated ($r = 0{\cdot}913$, $n = 70$) and had a slope of 59·8 mV per pH unit.

The effect of 5-hydroxytryptamine

Although we can still activate the Na^+–HCO_3^- co-transport in the absence of external K^+, we decided to use another method of changing the membrane potential in these glial cells to be sure that it was not the changes in the external K^+ concentration that were somehow directly altering the activity of the co-transporter.

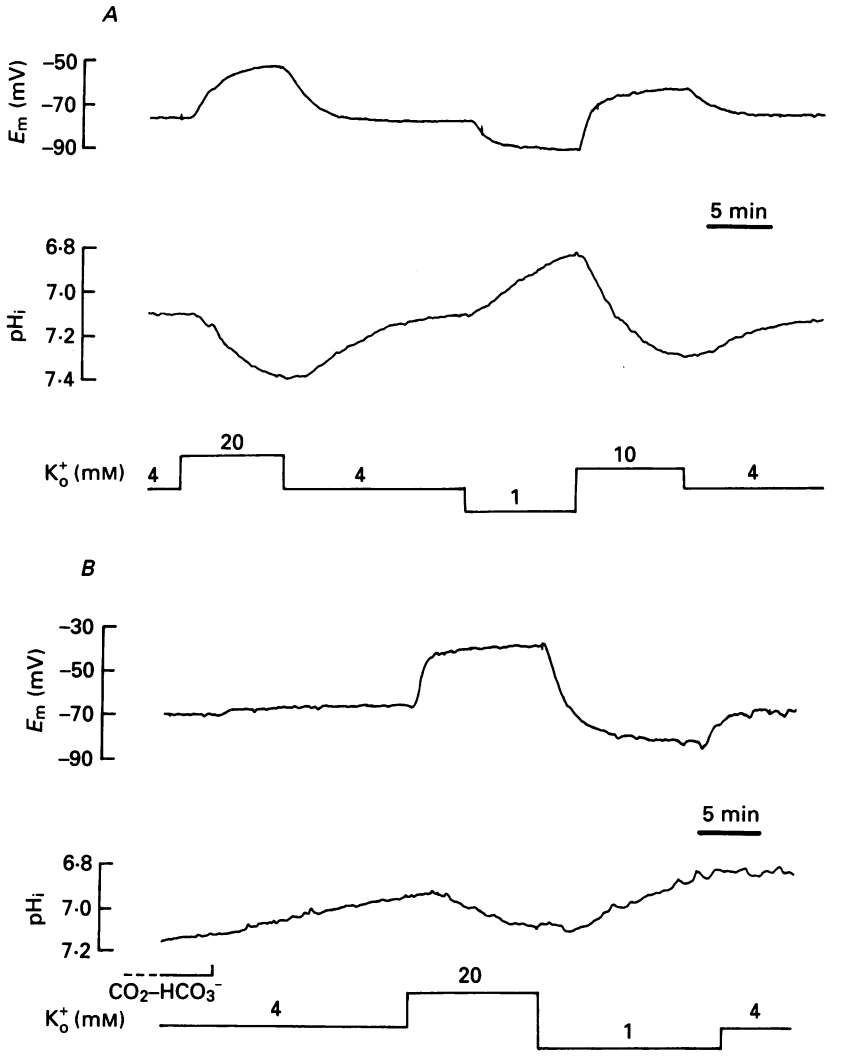

Fig. 2. The effect of different external K^+ concentrations between 1 and 20 mM on pH_i and E_m of a glial cell in the presence (*A*) and in the nominal absence (*B*) of 2% CO_2–11 mM-HCO_3^-.

The neurotransmitter 5-HT has been shown to hyperpolarize leech neuropile glial cells by increasing the K^+ permeability of the glial membrane (Walz & Schlue, 1982*a*). In a partially depolarized glial cell, 50 μM–5-HT was applied in the presence of CO_2–HCO_3^- (Fig. 4*A*). The membrane hyperpolarized from -50 to -80 mV, while the pH_i decreased from 7·5 to 7·2. Subsequent depolarization by increasing the external K^+ concentration was accompanied by a reversible increase of pH_i. In the nominal absence of CO_2–HCO_3^- a similar change in membrane potential by 5-HT changed the pH_i much more slowly and by usually less than 0·15 pH units (Fig. 4*B*).

Addition of 5-HT (50 or 100 μM) to glial cells with already high membrane

potentials, i.e. around −70 mV, resulted in very small (3 mV) changes in the membrane potential. Correspondingly, in these cells the pH_i changed by less than 0·1 pH units, indicating that it was not 5-HT itself which produced the pH_i changes.

Application of carbachol (0·1 mM), which depolarizes the glial cell membrane (Ballanyi & Schlue, 1988), resulted in a rapid intracellular alkalinization (not shown), dependent on the presence of CO_2–HCO_3^-. In contrast to 5-HT, however, carbachol itself, in the nominal absence of CO_2–HCO_3^-, produced an intracellular acidification.

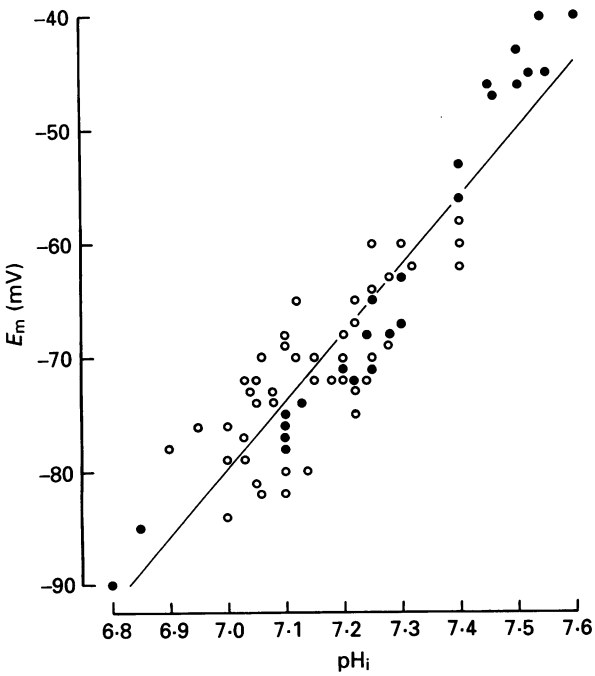

Fig. 3. Measurements of the steady-state pH_i of forty-five different glial cells in saline buffered with 2% CO_2–11 mM-HCO_3^- at normal external K^+ concentration (4 mM; ○) and at different external K^+ concentrations (like those shown in Fig. 2*A*) from eight cells (●) plotted *vs.* the membrane potential. The straight line is a least-squares regression fit through all points (coefficient of correlation $r = 0{\cdot}913$, $n = 70$) and has a slope of 59·8 mV/pH unit.

These experiments indicate that HCO_3^--dependent changes in pH_i can be simulated by different techniques which alter the membrane potential and further suggest that the pH_i shifts are directly linked to the changes in the membrane potential.

The Na^+ dependence of the depolarization-induced pH_i changes

If the depolarization-induced intracellular alkalinization is due to stimulation of electrogenic Na^+–HCO_3^- co-transport, it should be dependent on external Na^+. This is shown in Fig. 5. In the absence of external Na^+ (Fig. 5*A*) the introduction of CO_2–HCO_3^- produced an acidification, as expected from passive CO_2 entry into the cell. The lack of any larger pH_i change has also been observed previously in these

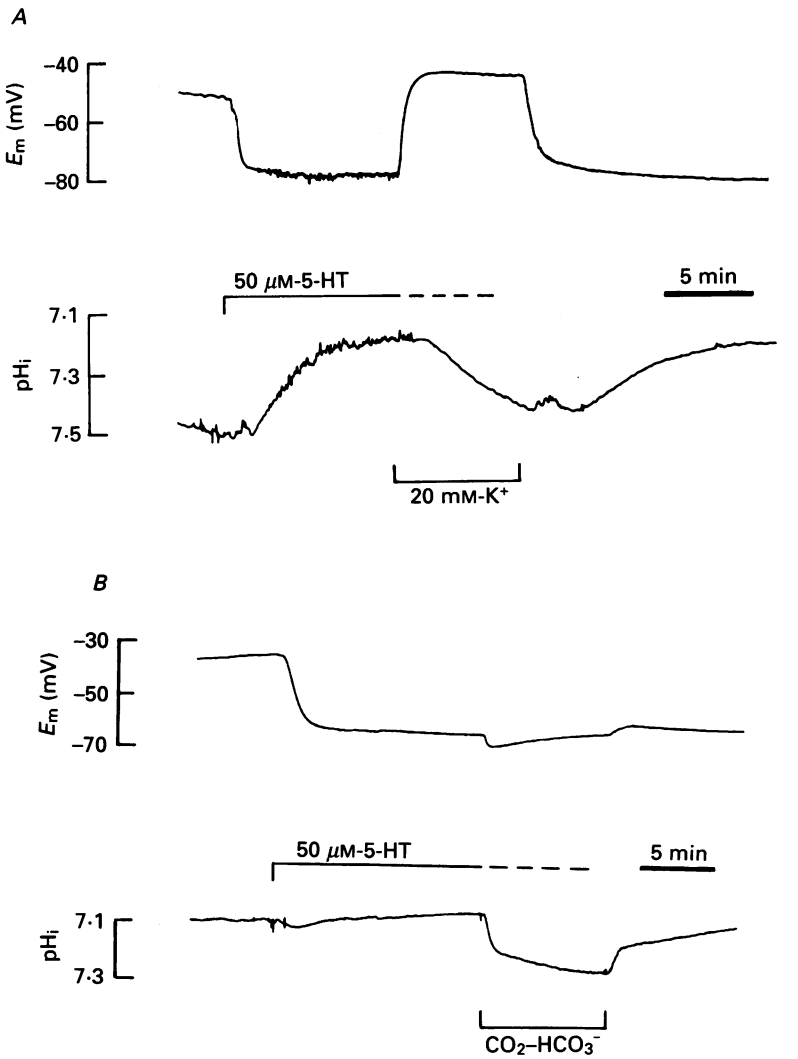

Fig. 4. Effect of the neurotransmitter 5-hydroxytryptamine (5-HT, 50 μM) and 20 mM-K^+ on pH_i and E_m in saline buffered with 2 % CO_2–11 mM-HCO_3^- (*A*) and the effect of 5-HT in the nominal absence of CO_2–HCO_3^- (*B*), both on partially depolarized neuropile glial cells.

cells (Deitmer & Schlue, 1989); the reason for this phenomenon is not yet fully understood. K^+-induced membrane depolarization in the absence of external Na^+ did not change pH_i significantly. Only when Na^+ was re-added to the saline in the presence of CO_2–HCO_3^-, did a marked increase of pH_i occur, accompanied by a prominent membrane hyperpolarization, presumably due to the activation of the electrogenic co-transporter. Increasing the external K^+ concentration to 20 mM now produced an additional intracellular alkalinization, from which the cell fully recovered as its membrane repolarized upon return to the normal leech saline.

In an experiment where external Na^+ was removed from a CO_2–HCO_3^--buffered saline solution containing 20 mM-K^+, a rapid intracellular acidification occurred, and the membrane depolarized by 15 mV (Fig. 5*B*). The speed of the decrease of pH_i and the membrane depolarization upon removal of external Na^+ suggested a reversal of

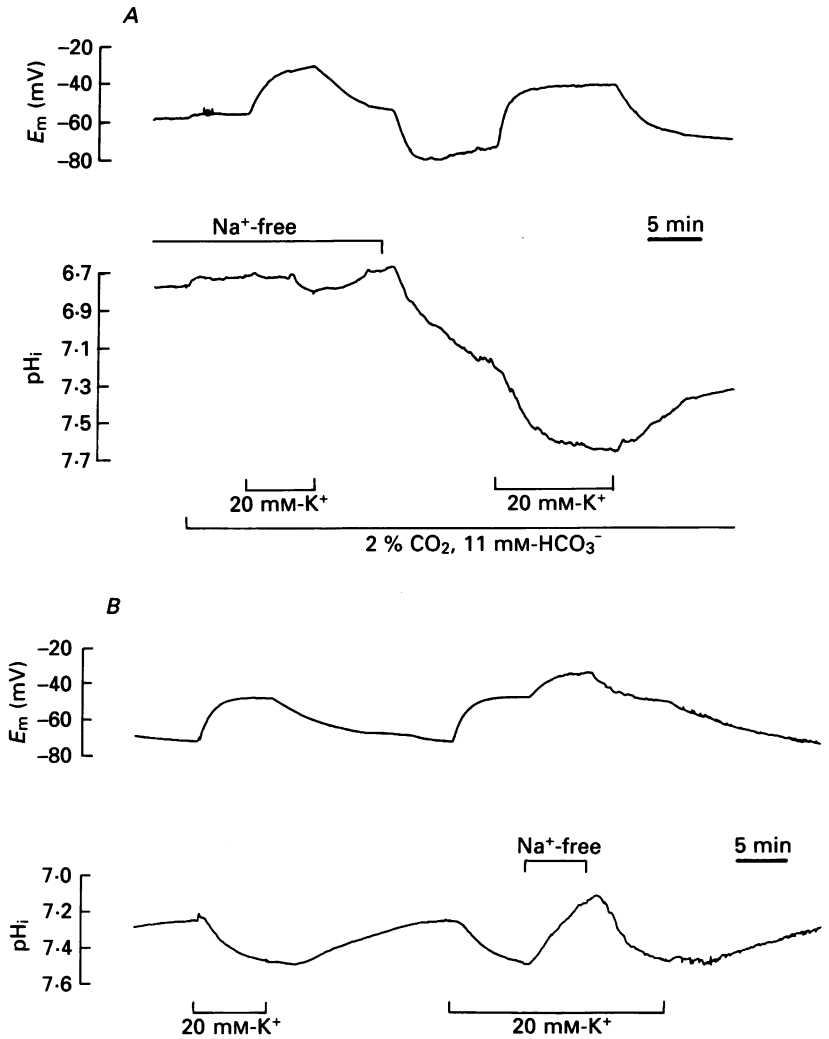

Fig. 5. *A*, the effect on pH_i and E_m of increasing the external K^+ concentration from 4 to 20 mM in the absence and presence of external Na^+ in a CO_2–HCO_3^--buffered saline. *B*, the effect of removal and re-addition of external Na^+ on pH_i and E_m in saline buffered with 2% CO_2–11 mM-HCO_3^- containing 20 mM-K^+.

Na^+–HCO_3^- co-transport, rather than acid influx and/or production of endogenous acid. Re-addition of external Na^+ produced a very rapid alkalinization at a rate of 0·06 pH units/min, accompanied by a membrane hyperpolarization, which is consistent with an activation of electrogenic Na^+–HCO_3^- co-transport. The subsequent decrease of the external K^+ concentration was followed by a membrane

hyperpolarization and a decrease of pH_i, both much slower than following removal of external Na^+.

The effect of DIDS

The intracellular alkalinization due to the K^+-induced membrane depolarization was inhibited by the stilbene DIDS. Addition of DIDS (0·5 mM) to a 20 mM-K^+-

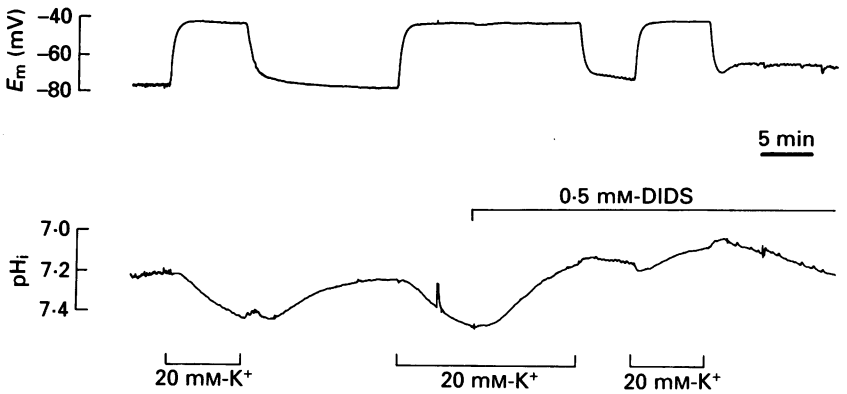

Fig. 6. The alkaline shift produced in saline buffered with 2% CO_2–11 mM-HCO_3^- in the presence of 20 mM-K^+ is reversed and inhibited after the addition of the stilbene (0·5 mM) to the saline.

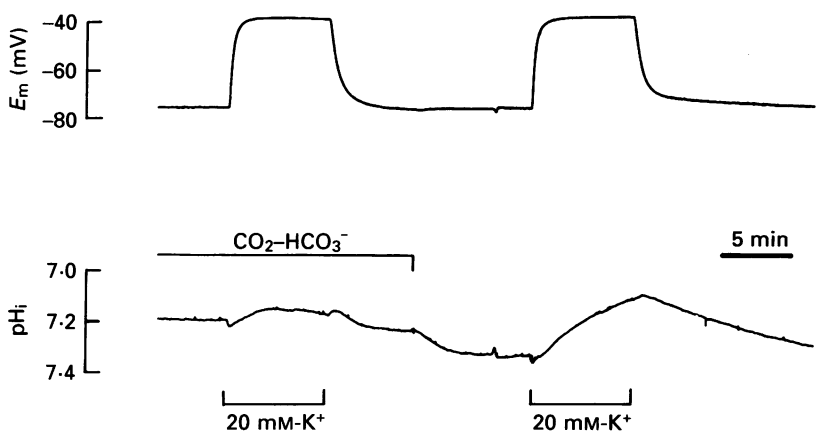

Fig. 7. The effect of increasing the external K^+ concentration from 4 to 20 mM in the presence and in the nominal absence of 2% CO_2–11 mM-HCO_3^- in a DIDS-poisoned glial cell.

containing, CO_2–HCO_3^--buffered saline solution (Fig. 6) in fact reversed this alkalinization and produced a rapid decrease of pH_i. Another exposure to 20 mM-K^+ in the continued presence of DIDS only produced a small transient pH_i change.

In other experiments of this kind, the Na^+–HCO_3^- co-transporter was fully

inhibited only after 3–8 min of DIDS application. In our experiments the effect of DIDS was partially reversible only after a short DIDS exposure (for less than 10 min). If DIDS was present for 15 min or longer, usually no reversibility of the inhibitory effect of DIDS was obtained.

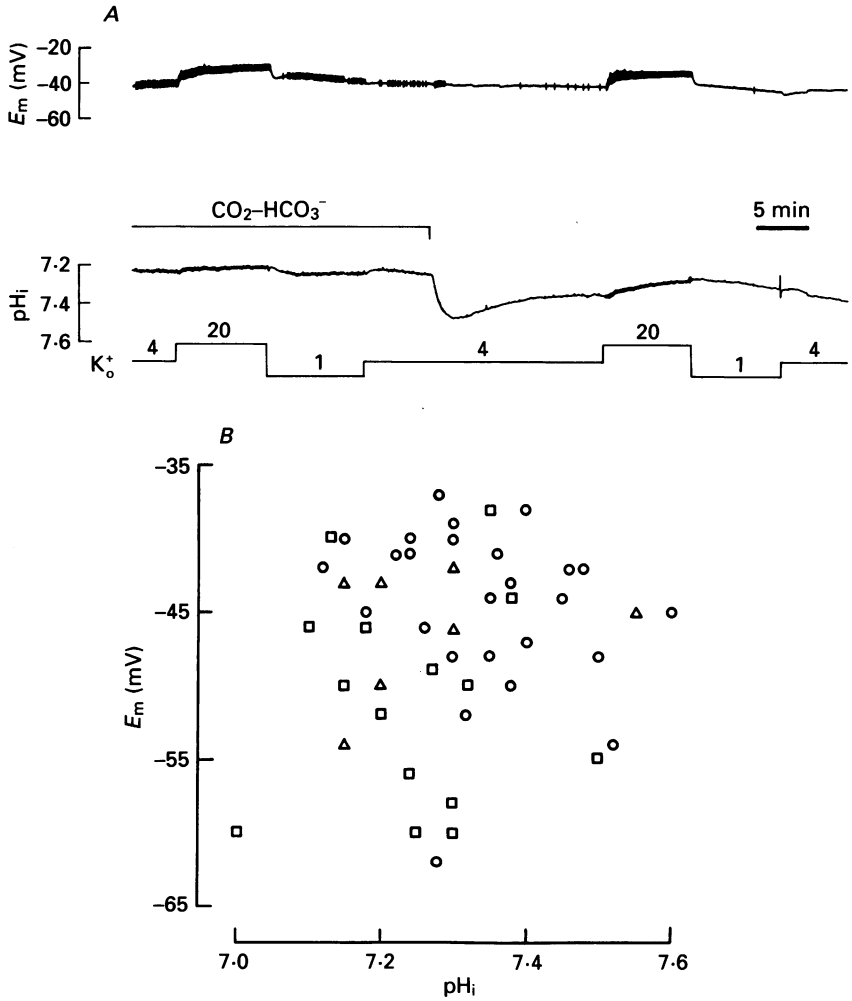

Fig. 8. *A*, measurements of pH_i and E_m of a Retzius neurone at different external K^+ concentrations between 1 and 20 mM in the presence and nominal absence of 2% CO_2–11 mM-HCO_3^-. *B*, plot of the measurements of pH_i and E_m of different identified neurones in CO_2–HCO_3^--buffered saline: Retzius cells (○), P (pressure) cells (□) and N (noxious) cells (△). There was no correlation between pH_i and E_m in these neurones ($r = 0{\cdot}155$, $n = 48$).

In a DIDS-poisoned preparation addition of CO_2–HCO_3^- produced a small acidification, which reversed, when CO_2–HCO_3^- was removed (Fig. 7), indicating that the Na^+–HCO_3^- co-transporter was indeed inhibited. Membrane depolarization in 20 mM-K^+ produced a small acidification in the presence of CO_2–HCO_3^-. However, in this DIDS-poisoned preparation, but in HEPES-buffered saline solution, 20 mM-

K^+ produced a larger decrease of pH_i with a maximum rate of 0·03 pH units/min (Fig. 7). The difference between the effect on the pH_i in the presence and absence of CO_2–HCO_3^- may partially be explained by assuming a higher buffer value of the cytoplasm in the presence of CO_2–HCO_3^-, and a HCO_3^--independent mechanism as a cause of the underlying intracellular acidification.

A comparison with the neuronal pH_i

In neurones, alteration of the external K^+ concentration produced a much smaller effect on the membrane potential (Deitmer & Schlue, 1981; Schlue & Deitmer, 1984). Increasing the external K^+ concentration from 4 to 20 mM depolarizes the membrane of a Retzius neurone by approximately 10 mV, and reduction of the external K^+ concentration to 1 or 0·2 mM often depolarizes the membrane by a few millivolts (presumably due to some inhibition of the electrogenic Na^+–K^+ pump in the Retzius cells, see Deitmer & Schlue, 1981). Changes of pH_i were measured at different external K^+ concentrations and membrane potentials and were found to be small in neurones ($< 0·1$ pH units), and as opposed to glial cells, even smaller in the presence than in the nominal absence of CO_2–HCO_3^- (Fig. 8*A*). The small intracellular acidifications after increasing the external K^+ concentration to 20 mM resembled the pH_i changes observed in DIDS-poisoned glial cells (see Fig. 7). The graph in Fig. 8*B* shows no correlation ($r < 0·16$, $n = 48$) between the membrane potential and the pH_i of leech neurones in the presence of CO_2–HCO_3^-. Indeed there is as yet no direct evidence, unlike in glial cells, of a Na^+–HCO_3^- co-transporter or any other electrogenic pH_i-regulating mechanism in leech neurones.

DISCUSSION

The main finding of this study is that the pH_i of leech neuropile glial cells, in the presence of CO_2–HCO_3^-, is strongly correlated with the membrane potential. The higher the membrane potential, the lower was the pH_i, and vice versa. The voltage-dependent changes in pH_i require the presence of external HCO_3^- and Na^+, and are inhibited by DIDS. These observations are consistent with the fact that electrogenic Na^+–HCO_3^- co-transport is a potent regulator of pH_i in these cells (Deitmer & Schlue, 1987, 1989).

Similar findings of voltage-dependent pH_i regulation as a consequence of electrogenic HCO_3^- transport have also been made in rat and in salamander proximal tubules (Alpern, 1985; Siebens & Boron, 1989), and in fused cells of the frog proximal tubule (Wang, Dietl, Silbernagl & Oberleithner, 1987).

The membrane potential of glial cells is believed to be largely due to the high K^+ permeability (Kuffler, Nicholls & Orkand, 1966; Gardner-Medwin, Coles & Tsacopoulos, 1981; Walz & Schlue, 1982*b*; Coles & Orkand, 1983; Gardner-Medwin, 1983; Kettenmann, Sonnhof & Schachner, 1983; Newman, 1986). The glial cell membrane is therefore very sensitive to changes in the extracellular K^+ level. We have used this glial property to shift the membrane potential to different values.

Another way to change glial membrane potential in the leech is to use neurotransmitters, such as 5-HT, which opens receptor-mediated K^+ channels in the glial cell membrane (Walz & Schlue, 1982*a*). Carbachol, an agonist of acetylcholine

receptors, has recently been shown to depolarize the glial membrane (Ballanyi & Schlue, 1988). Both substances not only change the membrane potential, but, in the presence of CO_2–HCO_3^-, also the pH_i in a similar way as changing the external K^+ concentration. We conclude from these results that it is the membrane potential itself which is the decisive parameter, directly changing the pH_i, and not K^+ or the neurotransmitters. Our conclusion is also confirmed by two further observations: first that an unpromoted membrane potential change, as e.g. a slow decrement of the membrane potential, is accompanied by an increase in pH_i (opposite to what one would expect from a dying cell), with both changes displaying the same time course; secondly that the co-transporter can be activated also in the absence of external K^+. Some modulating effect of external K^+ on the co-transporter, or a competition of external K^+ and Na^+, however, cannot as yet be excluded.

The relationship between pH_i and membrane potential in glial cells concluded from our experiments (Fig. 3) is that a depolarization of 60 mV should change the pH_i by one pH unit. Unfortunately a relationship of approximately 120 mV/pH unit is predicted from the proposed stoichiometry of 1:2 for Na^+–HCO_3^- co-transport in these cells. However, such a prediction assumes that in the steady state the co-transporter is in equilibrium. This is unlikely, since the glial cell possesses other pH_i- and intracellular Na^+-regulating mechanisms, and may also have pH_i-sensitive conductances. Furthermore, we have been unable to arrange a simple stoichiometry which predicts the co-transporter to be both at equilibrium in the steady state and give rise to a relationship of 60 mV/pH unit.

It is evident, however, from Fig. 2*B* that changes in the external K^+ concentration and hence membrane potential, also produce significant pH_i changes in the nominal absence of CO_2–HCO_3^-, although the rate and amount of the pH_i changes are reduced by 50% or more, as compared to those in the presence of CO_2–HCO_3^-. It should be emphasized that even in the nominal absence of CO_2–HCO_3^- the air CO_2 equilibrated in the HEPES-buffered saline solutions results in the formation of approximately 155 μM-HCO_3^-. In the case that the Na^+–HCO_3^- co-transporter has a high affinity for external HCO_3^-, the equilibrated HCO_3^- from air may activate the co-transporter to the extent indicated by the observed pH_i changes.

Our experiments show that in the presence of CO_2–HCO_3^- blockage of the Na^+–HCO_3^- co-transporter at raised external K^+ concentration, and therefore at high pH_i due to the stimulated co-transporter, results in a marked intracellular acidification. In cells where the pH_i is low, because the co-transporter has already been blocked either by removal of external Na^+ or application of DIDS, depolarization of the membrane has little or no effect on pH_i.

The dependence of pH_i on membrane potential may have a number of important implications. Even if membrane potential changes in glial cells are smaller and briefer under more physiological conditions than seen in our experiments, the resulting pH_i shifts could be quite significant, as seen e.g. in rat cortical astrocytes *in vivo* (Chesler & Kraig, 1987). These pH_i changes themselves may alter membrane conductances (Moody, 1984) and ion transport processes (Grinstein & Rothstein, 1986) across the glial membrane, besides influencing the activity of a variety of enzymes in the cell.

There is evidence that glial cells possess conductances and transport processes

other than a K^+ conductance, which may influence the membrane potential. Among these are a Cl^- conductance (Walz & Schlue, 1982*b*), electrogenic Na^+–K^+ pump (Grisar, Frere & Franck, 1979), and electrogenic sodium glutamate uptake (Brew & Atwell, 1987; Barbour, Brew & Atwell, 1988). Na^+–HCO_3^- co-transport is yet another electrogenic process the functioning of which will not only be voltage dependent, but may also itself contribute to the membrane potential. Indeed, stimulation of Na^+–HCO_3^- co-transport hyperpolarizes the glial membrane (Deitmer & Schlue, 1987, 1989, this study). The membrane depolarization obtained by raising the external K^+ levels was smaller by some millivolts in the presence of CO_2–HCO_3^- and Na^+, presumably due to stimulation of the Na^+–HCO_3^- co-transport (see Fig. 5). This may have further important physiological functions in the nervous system, especially when K^+ accumulates in the extracellular spaces (Somjen, 1979; Sykova, 1983).

The action of the Na^+–HCO_3^- co-transporter may have important functional consequences also with regards to the regulation of external K^+, which is considered to be one of the prime roles of glial cells. An alkalinization, which has been shown to occur in the presence of a high external K^+ concentration, has been demonstrated to increase the K^+ conductance in a number of preparations (Keller, Jentsch, Koch & Wiederholt, 1986; Henderson, Krampholz, Boyer & Graf, 1988) and in glial cells this may therefore facilitate K^+ uptake itself. An increase in gap junctional conductance upon intracellular alkalinization (Spray, Harris & Bennett, 1981) may promote the clearance of excess K^+ between neighbouring glial cells. Furthermore, due to a more negative membrane potential during stimulation of electrogenic Na^+–HCO_3^- co-transport, K^+ uptake by the glial cells would be further facilitated. Indeed we have measured the intracellular K^+ activity of these glial cells at elevated external K^+ concentrations and have found that intracellular K^+ rises to higher levels in the presence of CO_2–HCO_3^- than in its absence.

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REFERENCES

ALPERN, R. J. (1985). Mechanism of basolateral $H^+/OH^-/HCO_3^-$ transport in the rat proximal convoluted tubule: a sodium-coupled electrogenic process. *Journal of General Physiology* **86**, 613–636.

ASTION, M. L. & ORKAND, R. K. (1989). Electrogenic Na^+/HCO_3^- co-transport in neuroglia. *Glia* (in the Press).

BALLANYI, K. & SCHLUE, W. R. (1988). Direct effects of carbachol on membrane potential and ion activities in leech glial cells. *Glia* **1**, 165–167.

BARBOUR, B., BREW, H. & ATWELL, D. (1988). Electrogenic glutamate uptake in glial cells is activated by intracellular potassium. *Nature* **335**, 433–435.

BOURKE, R. S., KIMELBERG, H. K. & DAZE, M. A. (1978). Effects of inhibitors and adenosine on (HCO_3^-/CO_2)-stimulated swelling and Cl^- uptake in brain slices and cultured astrocytes. *Brain Research* **154**, 196–202.

BREW, H. & ATWELL, D. (1987). Electrogenic glutamate uptake is a major current carrier in the membrane of axolotl retinal glial cells. *Nature* **327**, 707–709.

CHESLER, M. & KRAIG, R. P. (1987). Intracellular pH of astrocytes increases rapidly with cortical stimulation. *American Journal of Physiology* **253**, R666–670.

COLES, J. A. & ORKAND, R. K. (1983). Modification of potassium movement through the retina of the drone (*Apis mellifera*) by glial uptake. *Journal of Physiology* **340**, 157–174.

DEITMER, J. W. & SCHLUE, W. R. (1981). Measurements of the intracellular potassium activity of Retzius cells in the leech central nervous system. *Journal of Experimental Biology* **91**, 87–101.

DEITMER, J. W. & SCHLUE, W. R. (1987). The regulation of intracellular pH by identified glial cells and neurones in the central nervous system of the leech. *Journal of Physiology* **388**, 261–283.

DEITMER, J. W. & SCHLUE, W. R. (1989). An inwardly directed electrogenic sodium–bicarbonate co-transport in leech glial cells. *Journal of Physiology* **411**, 179–194.

DEITMER, J. W. & SZATKOWSKI, M. S. (1989). Voltage-dependent regulation of intracellular pH is mediated by electrogenic Na^+–HCO_3^- co-transport in leech glia cells. *Journal of Physiology* **415**, 104*P*.

GARDNER-MEDWIN, A. R. (1983). A study of the mechanisms by which potassium moves through brain-tissue in the rat. *Journal of Physiology* **335**, 353–374.

GARDNER-MEDWIN, A. R., COLES, J. A. & TSACOPOULOS, M. (1981). Clearance of extracellular potassium: evidence for spatial buffering by glial cells in the retina of the drone. *Brain Research* **209**, 452–457.

GRINSTEIN, S. & ROTHSTEIN, A. (1986). Mechanisms of regulation of the Na^+/H^+ exchanger. *Journal of Membrane Biology* **90**, 1–12.

GRISAR, T., FRERE, J. M. & FRANCK, G. (1979). Effect of K^+ ions on kinetic properties of the (Na^+, K^+)-ATPase of bulk isolated glial cells, perikarya and synaptosomes from rabbit brain cortex. *Brain Research* **165**, 87–103.

HENDERSON, R. M., KRAMPHOLZ, B., BOYER, J. L. & GRAF, J. (1988). Effect of intracellular pH on potassium conductance in liver. *Pflügers Archiv* **412**, 334–335.

KELLER, S. K., JENTSCH, T. J., KOCH, M. & WIEDERHOLT, M. (1986). Interactions of pH and K^+ conductance in cultured bovine retinal pigment epithelial cells. *American Journal of Physiology* **250**, C124–137.

KETTENMANN, H. & SCHLUE, W. R. (1988). Intracellular pH regulation in cultured mouse oligodendrocytes. *Journal of Physiology* **406**, 147–162.

KETTENMANN, H., SONNHOF, U. & SCHACHNER, M. (1983). Exclusive potassium dependence of the membrane potential in cultured mouse oligodendrocytes. *Journal of Neuroscience* **3**, 500–505.

KIMELBERG, H. K., BIDDLECOME, S. & BOURKE, R. S. (1979). SITS-inhibitable Cl-transport and Na^+-dependent H^+ production in primary astroglial cultures. *Brain Research* **173**, 111–124.

KUFFLER, S. W. (1967). Neuroglial cells: Physiological properties and a potassium mediated effect of neuronal activity on the glial membrane potential. *Proceedings of the Royal Society* B **168**, 1–21.

KUFFLER, S. W., NICHOLLS, J. G. & ORKAND, R. K. (1966). Physiological properties of glial cells in the central nervous system of amphibia. *Journal of Neurophysiology* **29**, 768–787.

MOODY, W. L. (1984). Effects of intracellular H^+ on the electrical properties of excitable cells. *Annual Reviews of Neuroscience* **7**, 257–278.

NEWMAN, E. A. (1986). High potassium conductance in astrocyte endfeet. *Science* **233**, 453–454.

NICHOLSON, C. (1980). Dynamics of brain cell microenvironment. *Neuroscience Research Program Bulletin* **18**, 177–322.

ORKAND, R. K., COLES, J. A. & TSACOPOULOS, M. (1983). The role of glial cells in ion homeostasis in the retina of the honeybee drone. *Experimental Brain Research* **14**, 404–413.

SCHLUE, W. R. & DEITMER, J. W. (1984). Potassium distribution and membrane potential of sensory neurones in the leech central nervous system. *Journal of Neurophysiology* **51**, 689–704.

SCHLUE, W. R. & THOMAS, R. C. (1985). A dual mechanism for intracellular pH regulation by leech neurones. *Journal of Physiology* **364**, 327–338.

SCHLUE, W. R. & WUTTKE, W. (1983). Potassium in leech neuropile glial cells changes with external potassium concentration. *Brain Research* **270**, 368–272.

SIEBENS, A. W. & BORON, W. F. (1989). Depolarization-induced alkalinization in proximal tubules. I. Characteristics and dependence on Na^+. *American Journal of Physiology* **256**, F342–353.

SOMJEN, G. G. (1979). Extracellular potassium in the mammalian central nervous system. *Annual Review of Physiology* **41**, 159–177.

SPRAY, D. C., HARRIS, A. C. & BENNETT, M. V. L. (1981). Gap junctional conductance is a simple and sensitive function of intracellular pH. *Science* **211**, 712–715.

SYKOVA, E. (1983). Extracellular K^+ accumulation in the central nervous system. *Progress in Biophysics and Molecular Biology* **42**, 135–189.

WALZ, W. & SCHLUE, W. R. (1982*a*). Ionic mechanism of a hyperpolarizing 5-hydroxytryptamine effect on leech neuropile glial cells. *Brain Research* **250**, 111–121.

WALZ, W. & SCHLUE, W. R. (1982*b*). External ions and membrane potential of leech neuropile glial cells. *Brain Research* **239**, 119–138.

WANG, W., DIETL, P., SILBERNAGL, S. & OBERLEITHNER, H. (1987). Cell membrane potential: a signal to control intracellular pH and transepithelial hydrogen ion secretion in frog kidney. *Pflügers Archiv* **409**, 289–295.