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AN INWARDLY DIRECTED ELECTROGENIC SODIUM–BICARBONATE CO-TRANSPORT IN LEECH GLIAL CELLS

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SUMMARY

1. We have used double-barrelled ion-sensitive microelectrodes to measure the intracellular pH, pH_i, the intracellular Na^+ activity, a^i_{Na}, and the membrane potential in identified glial cells of the central nervous system of the leech *Hirudo medicinalis* to study the effect of CO_2–HCO_3^-.

2. When a HEPES-buffered saline was exchanged for a saline buffered with 2% CO_2 + 11 mM-HCO_3^-, keeping the pH constant at 7·4, the mean steady-state pH_i of the glial cells increased from 6·85 ± 0·06 to 7·18 ± 0·13 (mean ± S.D., $n = 25$).

3. This CO_2–HCO_3^--dependent alkalinization was inhibited in the absence of external Na^+ (exchanged by *N*-methyl-D-glucamine), but was unaffected by the inhibitor of Na^+–H^+ exchange, amiloride (2 mM).

4. The a^i_{Na} of the glial cells increased by 2–4 mM from a mean steady state of 7·2 ± 2 mM (mean ± S.D., $n = 6$) upon introduction of CO_2–HCO_3^--buffered saline. This CO_2–HCO_3^--dependent rise in a^i_{Na} increased to about double when the pH_i had been decreased by acid loading the cells (addition and subsequent removal of NH_4^+).

5. The CO_2–HCO_3^--dependent increases of pH_i and a^i_{Na} were inhibited by the stilbene 4,4-diisothiocyanostilbene-2,2′-disulphonic acid (DIDS, 0·5–1·0 mM).

6. Removal of external Cl^- and depletion of intracellular Cl^- did not inhibit the CO_2–HCO_3^--dependent alkalinization.

7. The CO_2–HCO_3^--dependent alkalinization was unaffected by inhibitors of the carbonic anhydrase, acetazolamide (0·2 mM) or ethoxzolamide (2 μM).

8. The membrane potential became more negative by 3–20 mV upon addition of CO_2–HCO_3^-. This hyperpolarization was even further enlarged in the presence of Ba^{2+} (which reduces the K^+ permeability) or at increased external K^+ concentration (which depolarizes the membrane and brings the membrane potential to the K^+ equilibrium potential). The CO_2–HCO_3^--induced membrane hyperpolarization was inhibited in Na^+-free saline and in the presence of DIDS. Ouabain (0·5 mM) sometimes reduced, but never abolished, the hyperpolarization.

9. The stoichiometry of the co-transport is suggested to be 2 HCO_3^-: 1 Na^+ with an equilibrium potential of −90 mV calculated for this coupling ratio in the steady state.

10. It is concluded that in the presence of CO_2–HCO_3^- an inwardly directed electrogenic Na^+–HCO_3^- co-transport is stimulated across the glial membrane, which

greatly determines the pH_i and thereby affects the intracellular buffering power of the glial cells.

INTRODUCTION

Glial cells are involved in the maintenance of ionic homeostasis in the central nervous system. Little is known, however, about the pH regulation by glial cells. Recently, we have reported direct measurements of the intracellular pH of identified glial cells in the central nervous system of the leech (Deitmer & Schlue, 1987*a*; Schlue & Deitmer, 1987). Our results indicated that in these glial cells in CO_2–HCO_3^--free, HEPES-buffered saline the pH is maintained at an alkaline value by an amiloride-sensitive Na^+–H^+ exchanger. In CO_2–HCO_3^--buffered saline there appeared two additional transport processes: one being sensitive to the stilbene SITS, (4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonic acid) and the other being unaffected by SITS and Na^+ dependent. The latter process shifted the intracellular pH of these glial cells by 0·3 pH units to more alkaline values.

In the present study we have looked at this CO_2–HCO_3^--dependent transport process in more detail. We have measured the intracellular pH, the intracellular Na^+ activity and the membrane potential in leech glial cells under various conditions. Our results suggest that in the presence of external CO_2–HCO_3^- an electrogenic and inwardly directed Na^+–HCO_3^- co-transport is stimulated. Some of the experiments have been reported in abstract form elsewhere (Deitmer & Schlue, 1987*b*, 1988).

METHODS

General

The experiments were performed on identified glial cells of the central nervous system of the medicinal leech *Hirudo medicinalis*. The preparations and dissection procedures used to isolate single ganglia have been described before (Schlue & Deitmer, 1980, 1984; Deitmer & Schlue, 1981). 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 less than 0·2 ml. The ganglia were superfused with saline at a rate of 15–20 bath volumes/min. The experiments were performed on the anterior and posterior glial cells and on packet glial cells. The selection and identification of glial cells essentially followed criteria described previously (Schlue, Schliep & Walz, 1980; Deitmer & Schlue, 1987*a*). All experiments were performed 3–8 times, each type with essentially similar protocols. The experiments were performed at room temperature (22–25 °C).

Physiological solutions

The normal leech saline (nominally HCO_3^--free) had the following composition (in mM): NaCl, 115; KCl, 4; $CaCl_2$, 1·8; HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid), 10; adjusted to pH 7·4 with NaOH. Solutions, buffered with CO_2–HCO_3^- instead of HEPES to pH 7·4, were equilibrated with nominally 2% CO_2/98% O_2 and 11 mM-$NaHCO_3$ (in which case NaCl was reduced to 114 mM). The solutions were then adjusted to pH 7·40 with NaOH or HCl, if needed. Ammonium-containing solutions were made by replacing 20 mM-NaCl with 20 mM-NH_4Cl, the NH_4Cl being added as a solid shortly before use. Cl^--free solutions were made by replacing Cl^- with gluconate.

In a series of experiments the carbonic anhydrase was inhibited by either ethoxzolamide (2 μM) or acetazolamide (0·2 mM). In other experiments DIDS (0·5 mM), the diuretics furosemide (1 mM) and amiloride (2 mM), and the glycoside ouabain (G-strophanthin, 0·5 mM) were directly added to the leech saline.

Microelectrodes

Double-barrelled ion-sensitive microelectrodes based on neutral carrier sensors selective for H^+

and for Na^+ were made similar to those described for K^+-sensitive microelectrodes (Schlue & Deitmer, 1980, 1984) and recently for pH-sensitive microlectrodes (Schlue & Thomas, 1985; Deitmer & Schlue, 1987*a*, *b*).

The pH-sensitive barrel contained at its tip the proton cocktail developed by Ammann, Lantern, Steiner, Schulthess, Shijo & Simon (1981), which we obtained from Fluka (Buchs, Switzerland). The reference barrel was filled with a solution of 3 M-KCl buffered with 10 mM-HEPES and adjusted to 7·0 with KOH, except for the experiments using Cl^--free solution, where it was filled with 3 M-lithium acetate, which had been found to have the least junction potential with respect to 3 M-KCl (Deitmer & Schlue, 1981). Details of the fabrication of the electrodes have been given previously (Deitmer & Schlue, 1987*a*). The electrodes were calibrated in leech salines buffered to 7·4 with 10 mM-HEPES, to 6·4 with 10 mM PIPES (piperazine-*N*,*N*′-bis(2-ethanesulphonic acid)) and to 8·0 with 10 mM-Tris (tris(hydroxymethyl)amino-methane).

The sensitivity of the electrodes to pH changes was dependent on the electrode tip size; the smaller the tip diameter, the lower its sensitivity. When the tip was broken to tip size greater than 2 μm, the electrodes usually responded with 58 mV/pH unit. In our experiments only electrodes giving a response of at least 45 mV/pH unit were accepted for experiments, the mean electrode response was 52 mV ($n = 50$). The sensitivity of the electrodes to CO_2–HCO_3^- was tested before and after each experiment. The response to CO_2–HCO_3^- could be transient and/or stepwise up to 0·15 pH units, when changing from CO_2–HCO_3^--free to 2% CO_2–11 mM-HCO_3^--containing leech saline (see Deitmer & Schlue, 1987*a*, *b*). When the electrode response to CO_2–HCO_3^- was larger by more than 2 mV either before or after the experiment, the recordings were discarded, and the electrode no longer used.

The Na^+-sensitive barrel contained at its tip the neutral carrier Na^+ ligand ETH 227 with tetraphenylborate (Steiner, Oehme, Ammann & Simon, 1979). The Na^+-sensitive microelectrode barrel was backfilled with 100 mM-NaCl + 10 mM-HEPES, pH 7·0, and the reference barrel with 3 M-KCl. The electrodes were calibrated in solutions with different Na^+ concentrations and a constant ionic background as described in Deitmer & Schlue (1983).

Recording

The electrical arrangements were the same as described previously (Schlue & Deitmer, 1980; Schlue & Thomas, 1985). Each channel of the double-barrelled microelectrode was connected to one input of a differential electrometer (WPI, F-223A) by chlorided silver wires. The bath electrode was a calomel electrode in 3 M-KCl connected to the bath by a 3 M-KCl–agar bridge. The electrometer outputs, the voltage of the ion-sensitive and the reference barrels, were displayed on an oscilloscope and recorded on a pen recorder (Gould 2400S).

RESULTS

The characteristic intracellular alkalinization monitored in neuropile glial cells upon introduction of a CO_2–HCO_3^--buffered saline is shown in Fig. 1. The intracellular alkalinization was maintained over 25 min. Hence, in CO_2–HCO_3^--buffered saline the steady-state pH_i was shifted from 7·02 to 7·23 in this experiment. The mean pH_i in HEPES-buffered saline was $6{\cdot}85 \pm 0{\cdot}06$ and in CO_2–HCO_3^--buffered saline $7{\cdot}18 \pm 0{\cdot}13$ (mean ± S.D., $n = 25$). Thus, in CO_2–HCO_3^--buffered saline the pH_i increased by an average of 0·33 pH units, a change which was fully reversible.

A similar alkalinization in CO_2–HCO_3^--buffered saline was also measured in packet glial cells, which surround the neuronal cell bodies in the ganglion periphery.

The Na^+ dependence of the alkalinization

In a previous paper (Fig. 7*A*, Deitmer & Schlue, 1987*a*) it was shown that the alkalinization did not occur when the HEPES-buffered saline was exchanged for a Na^+-free CO_2–HCO_3^--buffered saline. In the experiment shown in Fig. 2 we removed external Na^+ for 10 min which produced a small acidification of about 0·1 pH units

in the HEPES-buffered saline. Then the pH_i was allowed to stabilize before changing to a Na^+-free, CO_2–HCO_3^--buffered saline. The pH_i did not increase in this solution, but even slightly decreased. Hence, the CO_2–HCO_3^--dependent alkalinization is abolished in glial cells pre-incubated in Na^+-free saline.

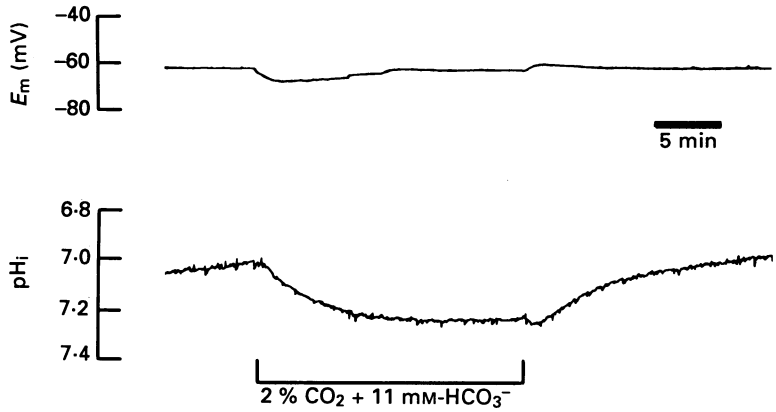

Fig. 1. Intracellular recording with a double-barrelled pH-sensitive microelectrode of pH (lower trace) and membrane potential E_m (upper trace) in a neuropile glial cell. A long exposure (25 min) to CO_2–HCO_3^--buffered saline produced an alkaline shift which is maintained over the period of CO_2–HCO_3^- presence and fully reversible after removal of CO_2–HCO_3^-.

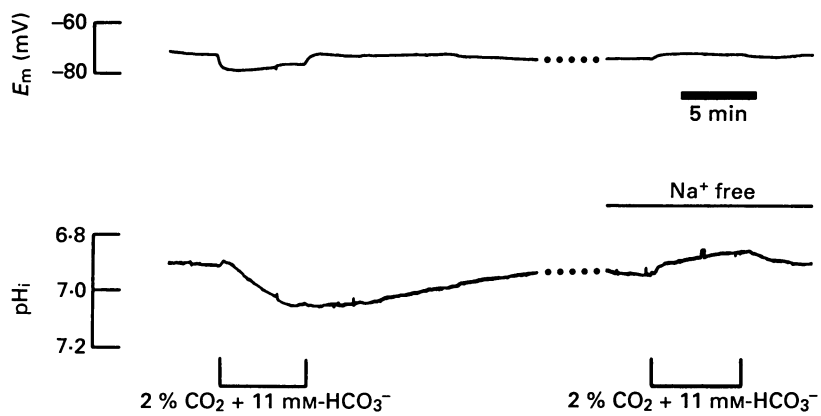

Fig. 2. Inhibition of the intracellular alkalinization and membrane hyperpolarization induced by CO_2–HCO_3^- in Na^+-free saline. External Na^+ was exchanged 10 min before the second exposure to CO_2–HCO_3^- by 130 mM-*N*-methyl-D-glucamine.

When Na^+ was removed in the presence of CO_2–HCO_3^-, the pH_i decreased rapidly (and the membrane depolarized). This was also observed in connective glial cells of the leech (M. Szatkowski, personal communication), and suggests the reversal of the process studied here.

The effect of amiloride

The Na^+ dependence of the CO_2–HCO_3^--induced alkalinization may be due to stimulation of the Na^+–H^+ exchanger, which was shown to be present in these glial cells (Deitmer & Schlue, 1987*a*). We therefore studied the effect of amiloride, a well-known inhibitor of Na^+–H^+ exchange (Bentley, 1968; Frelin, Vigne, Barbry & Lazdunski, 1987), on the CO_2–HCO_3^--induced alkalinization (Fig. 3). Addition of

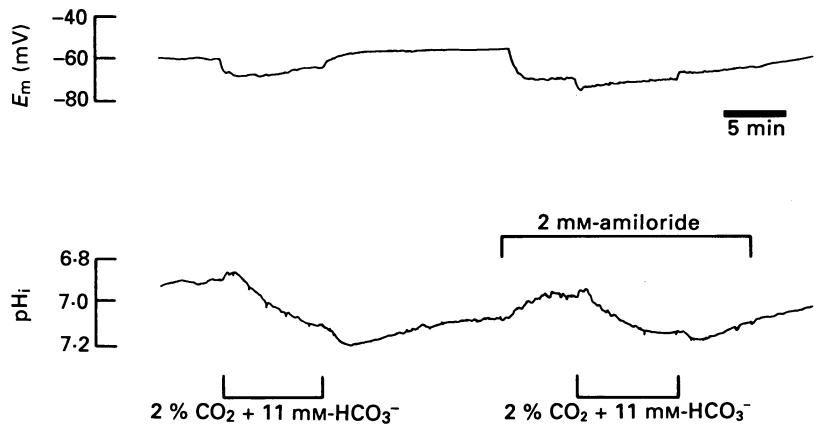

Fig. 3. The effect of the K^+-sparing diuretic amiloride (2 mM) on the CO_2–HCO_3^--induced intracellular alkalinization.

amiloride (2 mM) produced a small acidification of about 0·1 pH units which might be due to inhibition of Na^+–H^+ exchange. Amiloride did not, however, interfere with the CO_2–HCO_3^--induced alkalinization. This suggests that the activity of the Na^+–H^+ exchanger does not make a significant contribution to the CO_2–HCO_3^--induced alkalinization.

Measurements of the intracellular Na^+ activity

An important question was whether the HCO_3^--induced alkalinization was not only Na^+-dependent, but also accompanied by a Na^+ influx. If the process underlying the alkalinization was a Na^+–HCO_3^- co-transport, as suggested previously (Deitmer & Schlue, 1987*a*), a rise of intracellular Na^+ should be observed upon addition of CO_2–HCO_3^-. We therefore measured the intracellular Na^+ activity, a^i_{Na}, directly. In the glial cell shown in Fig. 4 the resting a^i_{Na} was 6·5 mM in HEPES-buffered saline. On average the a^i_{Na} of the glial cells was $7{\cdot}2 \pm 2$ mM ($n = 6$).

When the HEPES-buffered saline was twice replaced by a CO_2–HCO_3^--buffered saline, a^i_{Na} increased by 2·5 and 3 mM, respectively. The a^i_{Na} increase was rapid and sometimes began to reverse even during the presence of CO_2–HCO_3^-. Upon removal of CO_2–HCO_3^- the a^i_{Na} returned to its initial level with increased rate. In three other experiments of this kind the CO_2–HCO_3^--dependent rise of a^i_{Na} ranged between 2 and 4 mM.

It would be expected that the driving force for HCO_3^- influx might be increased if pH_i was more acid, thereby increasing the rate of Na^+–HCO_3^- co-transport. The effect of intracellular acidification on the CO_2–HCO_3^--dependent increase of a^i_{Na} is shown in Fig. 5. After the acid load by the NH_4^+-pre-pulse technique the CO_2–HCO_3^--induced rise of a^i_{Na} was more than twice as large: a^i_{Na} increased from 6 to 13 mM as compared

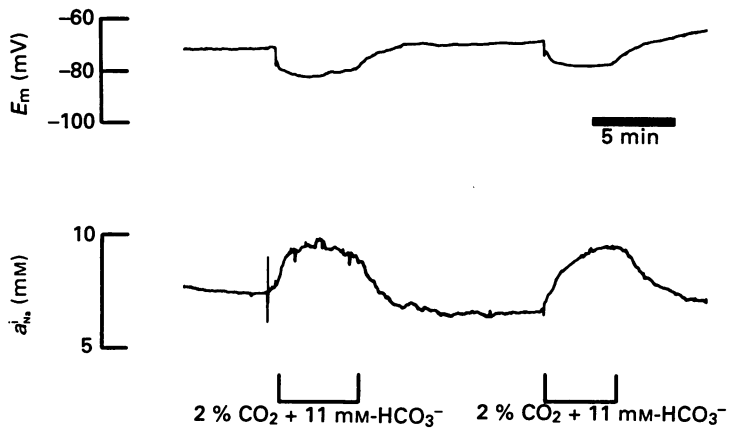

Fig. 4. Measurements of the intracellular Na^+ activity, a^i_{Na}, (lower trace) and membrane potential (upper trace). Two exposures to CO_2–HCO_3^--buffered saline each induced a rise in a^i_{Na} of several millimolar.

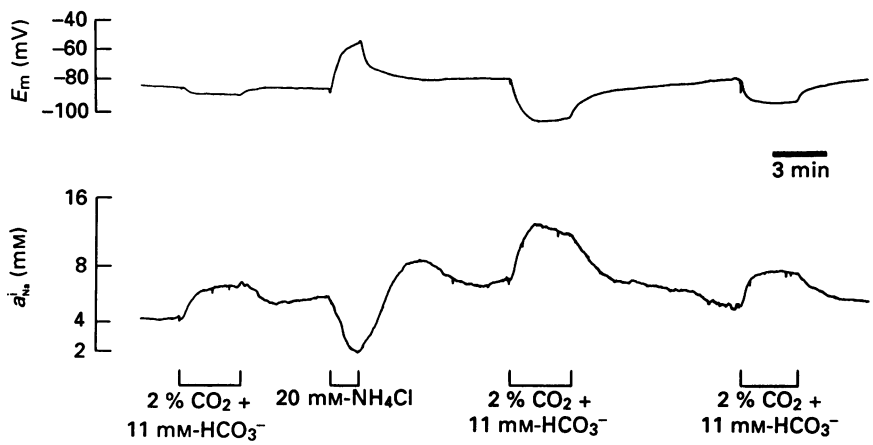

Fig. 5. Increase of the CO_2–HCO_3^--induced rise in a^i_{Na} before, during and after intracellular acidification produced by addition and removal of NH_4Cl.

to the rise from 4 to 6·5 mM before the acid load. From our pH_i measurements we know that, following a NH_4^- pre-pulse, the pH_i of glial cells decreases within 3–5 min to its lowest value and recovers within 10–20 min to its initial level (Deitmer & Schlue, 1987*a*). After 10 min, when the pH_i was assumed to have recovered by a considerable amount from the previous acid load, exposure to CO_2–HCO_3^- increased a^i_{Na} by an amount similar to that during the first control exposure before application of NH_4Cl.

The effect of DIDS on pH_i and a^i_{Na} changes

The stilbene DIDS has been shown in a variety of cells to block Na^+–HCO_3^- co-transport (Boron & Boulpaep, 1983; Jentsch, Schill, Schwartz, Matthes, Keller & Wiederholt, 1985). We used DIDS (0·5 mM) on the CO_2–HCO_3^--induced increase of pH_i and a^i_{Na} (Fig. 6). It is clearly shown that DIDS completely blocked the

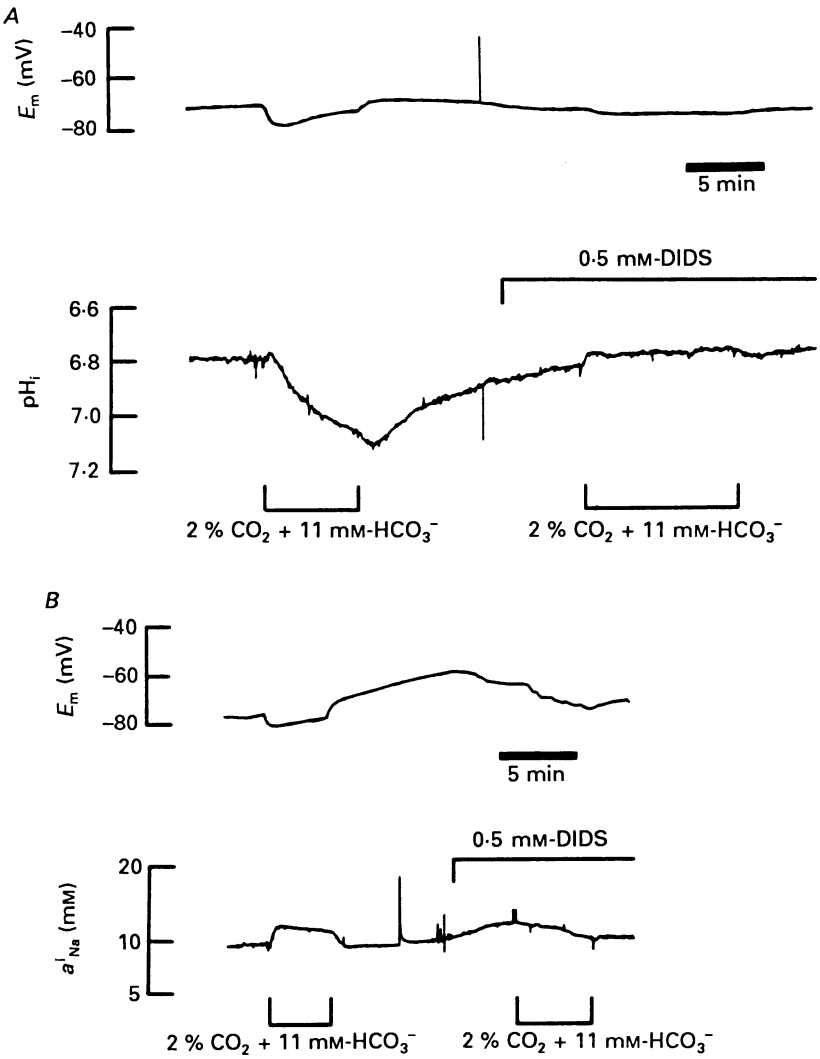

Fig. 6. The action of the stilbene DIDS (0·5 mM) on the membrane hyperpolarization and on the change of pH_i (*A*) and of a^i_{Na} (*B*) induced by CO_2–HCO_3^-.

alkalinization (Fig. 6*A*), and the rise of a^i_{Na} (Fig. 6*B*). In the presence of DIDS, CO_2–HCO_3^- produced a small reversible intracellular acidifcation, similar to that observed in Na^+-free saline (Fig. 2). There was a small rise of a^i_{Na} after addition of DIDS, which appeared to reverse slowly even during the presence of DIDS.

The effect of Cl^- removal

In neurones a Na^+- and HCO_3^--dependent exchange process has been described which also shows a dependence on Cl^-. This was first reported for squid axons

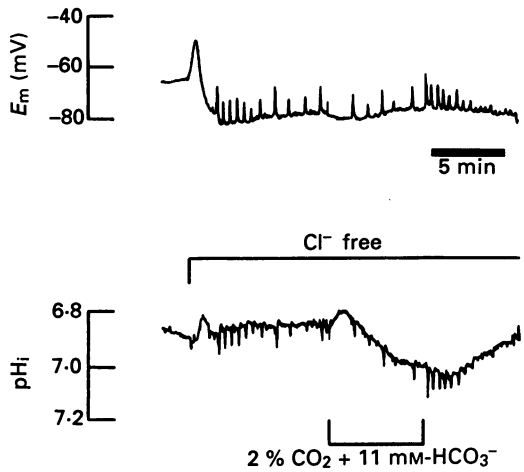

Fig. 7. The CO_2–HCO_3^--induced change in pH_i in a saline incubated in a saline where Cl^- was exchanged by gluconate. The upward deflections on the membrane potential recording (upper trace) are slow depolarizations of the glial cell membrane.

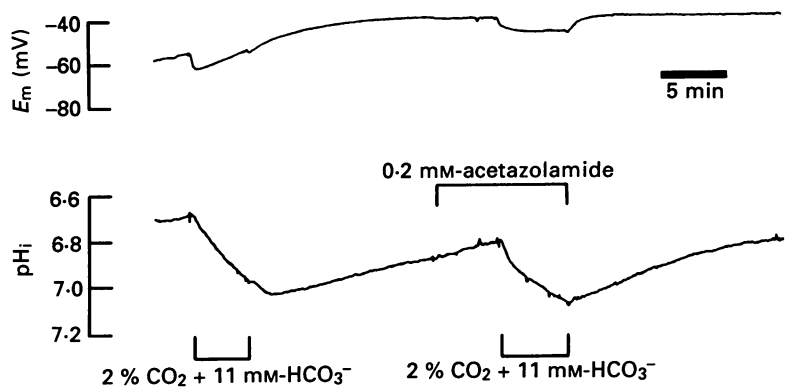

Fig. 8. The effect of the inhibitor of carbonic anhydrase, acetazolamide (0·2 mM), on the CO_2–HCO_3^--induced change in pH_i and membrane potential.

(Russell & Boron, 1976) and for snail neurones (Thomas, 1977), as a Na^+–H^+–Cl^-–HCO_3^- transporter, which extrudes acid and Cl^- and promotes Na^+ and HCO_3^- influx. It seems essential, therefore, to establish the role of Cl^- during the CO_2–HCO_3^--induced glial alkalinization. After pre-incubation in Cl^--free saline for 10 min, addition of CO_2–HCO_3^- still produced an alkalinization by 0·2 pH units

(Fig. 7). Experiments using Cl^--sensitive microelectrodes showed that the intracellular Cl^- depleted fully within about 5 min in these glial cells (K. Ballanyi, personal communication). It is concluded from these experiments that Cl^- ions are not involved in the process underlying the CO_2–HCO_3^--induced intracellular alkalinization.

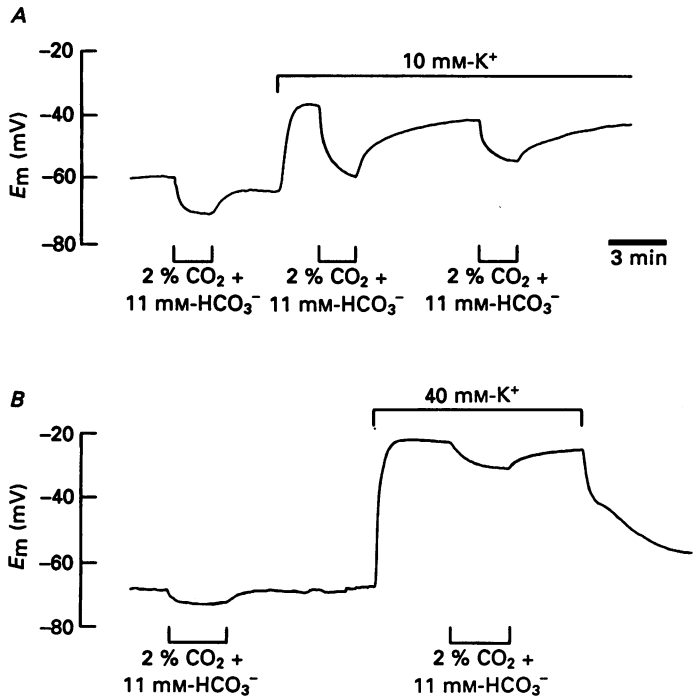

Fig. 9. Membrane potential recordings during short exposures to CO_2–HCO_3^--buffered saline in normal (4 mM) and elevated K^+ (10 mM in *A*, 40 mM in *B*) in the external solution.

The effect of carbonic anhydrase inhibitors

We have tested the carbonic anhydrase inhibitors acetazolamide and ethoxzolamide on the CO_2–HCO_3^--induced alkaline shift in the glial cells. The alkaline shift produced by CO_2–HCO_3^- was not affected by 0·2 mM-acetazolamide (Fig. 8). This inhibitor concentration completely abolished the transient acidification upon addition of CO_2–HCO_3^-, and the transient alkalinization upon removal of CO_2–HCO_3^- in Retzius neurones (not shown). Ethoxzolamide (2 μM), another potent inhibitor of CO_2–HCO_3^--induced transient pH_i changes in neurones ($K_{0.5} \sim 5 \times 10^{-8}$ M, J. W. Deitmer & W.-R. Schlue, unpublished observation), also had no effect on the CO_2–HCO_3^--induced alkalinization in glial cells. Thus, the activity of a carbonic anhydrase was not directly involved in the glial alkalinization.

CO_2–HCO_3^--induced membrane potential changes

A change to the CO_2–HCO_3^--buffered saline produced a membrane hyperpolarization, which was sometimes partly transient, but often sustained over the period of CO_2–HCO_3^- presence. This hyperpolarization appeared to be associated

with the increases of pH_i and a^i_{Na}, since it was inhibited in Na^+-free saline and by DIDS (Figs 2 and 6). There were two possible mechanisms underlying the hyperpolarization of the glial cells: (1) a change in membrane conductance, as for example an increase in the K^+ permeability, and/or (2) stimulation of an electrogenic process.

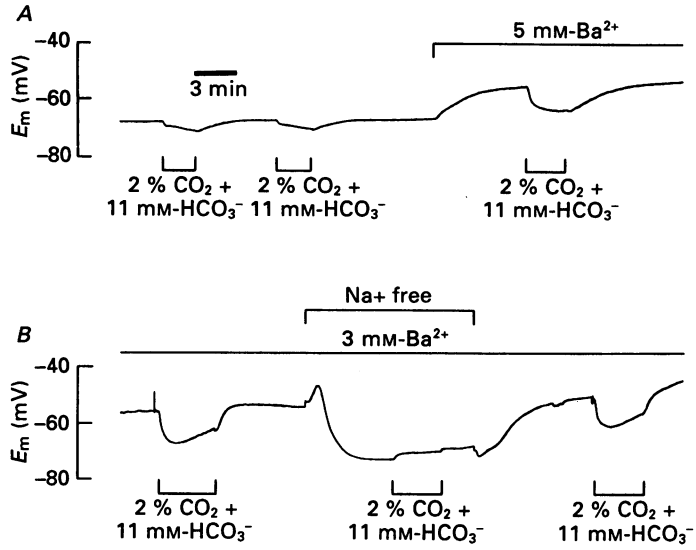

Fig. 10. The effect of Ba^{2+} on the membrane potential and on the CO_2–HCO_3^--induced hyperpolarization (*A*) in the presence and absence of external Na^+ (*B*).

Effect of raised K^+

We measured the membrane potential of neuropile glial cells with conventional, single-barrelled microelectrodes, and we recorded the CO_2–HCO_3^--induced membrane potential change in the presence of 10 mM-K^+ and 40 mM-K^+ (Fig. 9). The membrane potential in normal saline (4 mM-K^+) was −60 mV in the experiment shown in Fig. 9*A*, and it reversibly became more negative by 12 mV in the presence of CO_2–HCO_3^-. In 10 mM-K^+, the membrane depolarized to −40 mV, and two subsequent exposures to CO_2–HCO_3^- at this raised K^+ concentration produced hyperpolarizations of 22 and 13 mV.

In an experiment with 40 mM-K^+ (Fig. 9*B*) CO_2–HCO_3^- evoked a hyperpolarization of 8 mV, as compared to 5 mV in 4 mM-K^+. Since the membrane potential of these glial cells is virtually identical with the K^+ equilibrium potential in a saline with an increased K^+ concentration (Walz & Schlue, 1982; Wuttke, 1986), we conclude that the CO_2–HCO_3^--induced hyperpolarization is not due to a change in the membrane K^+ conductance. These experiments also suggest that the hyperpolarization is not due to reduction of a Na^+ conductance, since this would not bring the membrane potential to values more negative than the K^+ equilibrium potential. A change in the Cl^- conductance could be excluded, because the Cl^- distribution in neuropile glial cells is passive, and the Cl^- electrochemical equilibrium potential follows the membrane potential (Wuttke, 1986; K. Ballanyi, personal communication).

Effects of Ba^{2+}

The CO_2–HCO_3^--induced membrane hyperpolarization was also studied in the presence of Ba^{2+}, which reduces the K^+ permeability of the cell membrane. Addition of 5 mM-Ba^{2+} depolarized the glial membrane in the experiment shown in Fig. 10*A* from −65 to −55 mV. The hyperpolarization upon exposure to a CO_2–HCO_3^--buffered saline, however, increased from 3–4 to 10 mV in the presence of Ba^{2+}. The larger membrane potential change is probably due to the increased membrane resistance produced by Ba^{2+}. In another experiment using 3 mM-Ba^{2+} the CO_2–HCO_3^--induced hyperpolarization amounted to 12 mV (Fig. 10*B*). After the

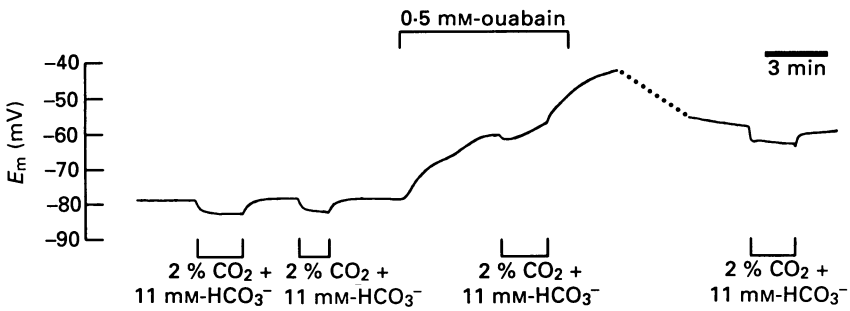

Fig. 11. The effect of ouabain (0·5 mM) on the CO_2–HCO_3^--induced membrane hyperpolarization. The dotted line indicates a period of 20 min.

removal of external Na^+, the membrane hyperpolarized to −75 mV, and the addition of CO_2–HCO_3^- produced a small depolarization. Following readdition of external Na^+, the membrane depolarized to −50 mV, and CO_2–HCO_3^- again produced a membrane hyperpolarization of 10 mV. This confirms that the CO_2–HCO_3^--induced membrane hyperpolarization was Na^+-dependent, and unlikely to be due to an increase in membrane K^+ conductance.

Effect of ouabain

In the experiment shown in Fig. 5, the membrane potential was driven to −101 mV following the addition of CO_2–HCO_3^- during an acid load and an increased rise in the a^i_{Na}. This value is more negative than all ionic equilibrium potentials. The experiment in Fig. 5 also pointed to the possibility that the CO_2–HCO_3^--induced hyperpolarization was related to the increase of a^i_{Na}, and hence due to an increased activity of an electrogenic Na^+-K^+ pump. We therefore studied the effect of ouabain on the CO_2–HCO_3^--induced membrane hyperpolarization. Ouabain (0·5 mM) did not block this hyperpolarization (Fig. 11). In the experiment shown the membrane hyperpolarization induced by CO_2–HCO_3^- was 4 mV before, during, and 20 min after the exposure to ouabain. Ouabain itself depolarized the membrane (see also Walz, Wuttke & Schlue, 1983). In two out of five experiments, however, ouabain reversibly reduced the hyperpolarization by up to 50%.

These experiments show that during the rise of a^i_{Na} an electrogenic Na^+-K^+ pump might be stimulated which contributed to the hyperpolarization. However, there always remained a ouabain-resistant hyperpolarization. It is therefore concluded that this remaining membrane hyperpolarization is due to the coupled influx of Na^+ and HCO_3^-, and hence indicative for the electrogenicity of the Na^+–HCO_3^- co-transport. The direction of the membrane potential change infers that more HCO_3^- ions are transported into the glial cells than Na^+ ions during this process.

Stoichiometry of the co-transport

Given the directly measured activity of a^i_{Na}, being 7·2 mM, and the mean value for the intracellular HCO_3^- concentration of 6·6 mM obtained from the pH_i measurements, and assuming a Na^+ activity coefficient to be 0·75, the equilibrium potential of the Na^+–HCO_3^- co-transport, E_{NaHCO_3}, can be calculated according to the equation (see also Boron & Boulpaep, 1983):

$$E_{NaHCO_3} = \frac{RT}{zF} \ln \frac{[Na^+]_o [HCO_3^-]_o^n}{[Na^+]_i [HCO_3^-]_i^n}, \quad (1)$$

where n is the HCO_3^-: Na^+ stoichiometry and R, T, F and z have their usual meanings.

The E_{NaHCO_3}, where no net ion transport is mediated by the carrier, was calculated to be −90 mV for $n = 2$, and −52 mV for $n = 3$ at steady-state conditions. Since a membrane hyperpolarization was recorded at all membrane potentials between −25 mV (Fig. 9*B*), and −83 mV (Fig. 5), which was partly maintained throughout the experiment, and a more negative steady-state membrane potential of −72 mV was measured in CO_2–HCO_3^--buffered saline as opposed to in HEPES-buffered saline, the coupling ratio must be less than 3:1. Hence, in leech glial cells the co-transport presumably carries 2 HCO_3^- ions with 1 Na^+ ion.

DISCUSSION

The present study provides evidence for the existence of a Na^+–HCO_3^- co-transport in the membrane of glial cells of the leech central nervous system. This co-transport is directed inwardly, i.e. producing an influx of Na^+ and HCO_3^- ions into the glial cells, and it is electrogenic by transporting more HCO_3^- than Na^+ ions. The main evidence is the following: (1) addition of CO_2–HCO_3^- as external buffer produced an intracellular alkalinization (indicative of an increase of intracellular HCO_3^-), a rise of intracellular Na^+, and a membrane hyperpolarization; (2) removal of either HCO_3^- or Na^+ from the saline, or the addition of the stilbene DIDS, abolished or reversed the increases of pH_i and a^i_{Na}, and the membrane hyperpolarization; (3) the CO_2–HCO_3^--induced alkalinization was unaffected by amiloride, an inhibitor of Na^+–H^+ exchange, and by the depletion of external and intracellular Cl^-. In addition, the CO_2–HCO_3^--induced changes in pH_i and membrane potential were not affected by inhibitors of the carbonic anhydrase. The Na^+- and HCO_3^--dependent membrane hyperpolarization was not due to a change of the membrane conductance, and was only partly ouabain sensitive. This strongly suggests that the glial Na^+–HCO_3^- transport is electrogenic, presumably carrying 2 HCO_3^- ions with 1 Na^+ ion.

Na^+–HCO_3^- co-transport in epithelial and glial cells

A Na^+–HCO_3^- co-transport has been described in epithelial cells, such as amphibian and mammalian kidney (Boron & Boulpaep, 1983; Alpern, 1985; Jentsch *et al.* 1985; Jentsch, Matthes, Keller & Wiederholt, 1986; Yoshitomi, Burckhardt & Frömter, 1985; Soleimani, Grassl & Aronson, 1987), mammalian corneal endothelium (Jentsch, Keller, Koch & Wiederholt, 1984) and amphibian stomach (Curci, Debellis & Frömter, 1987). A similar transport mechanism has recently been suggested also for cultured mouse oligodendrocytes (Kettenmann & Schlue, 1988). In mammalian smooth muscle cells (Aickin, 1984) CO_2–HCO_3^- induced an alkalinization similar to that observed in glial cells.

The Na^+–HCO_3^- co-transport in leech glial cells differs from the Na^+–HCO_3^- co-transport in intact epithelial cells in that there the movement of Na^+ and HCO_3^- at the basolateral membrane is *out* of the cell (Boron & Boulpaep, 1983; Yoshitomi *et al.* 1985; Curci *et al.* 1987). In leech glial cells this process is *inwardly* directed, i.e. Na^+ and HCO_3^- move into the cell. This has important consequences; while the outwardly directed co-transport in epithelial cells must be electrogenic to use the negative membrane potential to drive Na^+ with HCO_3^- out of the cell, an inwardly directed co-transport may use the Na^+ gradient to drag HCO_3^- into the cell.

Another important difference between the Na^+–HCO_3^- co-transport in vertebrate epithelial cells and leech glial cells results from the dependence of the outwardly directed transport in epithelia on *intracellular* HCO_3^-. Hence, Na^+–HCO_3^- co-transport occurs only in the presence of CO_2–HCO_3^- in the saline, as is also the case in glial cells.

The Na^+–HCO_3^- co-transport is reduced by carbonic anhydrase inhibitors in epithelial cells (and it is the activity of the carbonic anhydrase which rapidly converts CO_2 and H_2O into H^+ and HCO_3^-). While H^+ ions are extruded by the Na^+–H^+ exchanger (for references see Aronson, 1985, and Grinstein & Rothstein, 1986), HCO_3^- can be used as substrate for Na^+–HCO_3^- co-transport.

The present experiments have clearly shown that the carbonic anhydrase is not involved in Na^+–HCO_3^- co-transport in leech glial cells. It appears as if leech neuropile glial cells may have a low activity of carbonic anhydrase. It remains to be clarified, however, why carbonic anhydrase inhibitors inhibit Na^+–HCO_3^- co-transport in epithelial cells but do not affect the Na^+–HCO_3^- co-transport in these glial cells.

The CO_2–HCO_3^--induced membrane hyperpolarization

Our experiments have shown that the membrane hyperpolarization which accompanies the CO_2–HCO_3^--induced changes in pH_i and a^i_{Na} is not due to an increase of K^+ conductance. The presence of CO_2–HCO_3^- may, however, stimulate a HCO_3^- permeability of the membrane, as was recently suggested for the glial cell in *Necturus* optic nerve (Astion, Coles & Orkand, 1987). Due to the HCO_3^- distribution in leech glial cells and the calculated HCO_3^- equilibrium potential of -13 mV (see also Deitmer & Schlue, 1987*a*), a HCO_3^- permeability increase would *depolarize* the cell membrane. A transient hyperpolarization would be expected to occur only during the introduction of HCO_3^-, which would then reverse into a depolarization. Although

the CO_2–HCO_3^--induced hyperpolarization was often, but not always, partly transient, a depolarization was never observed. It is therefore unlikely that a HCO_3^- permeability can account for the CO_2–HCO_3^--induced membrane hyperpolarization.

The hyperpolarization was believed to be due to an electrogenic process. Since the inhibitor of the Na^+–K^+ pump, ouabain, did not abolish the hyperpolarization, we conclude that it is the Na^+–HCO_3^- co-transport itself which is electrogenic. In vertebrate epithelial cells Na^+–HCO_3^- co-transport was reported to be electrogenic (Boron & Boulpaep, 1983; Yoshitomi *et al.* 1985; Jentsch *et al.* 1985, 1986).

A stoichiometry of 2 HCO_3^-:1 Na^+ was suggested for the Na^+–HCO_3^- co-transport in renal proximal tubule of the salamander (Boron & Boulpaep, 1983), while a stoichiometry of 3:1 was favoured in mammalian renal epithelia (Yoshitomi *et al.* 1985; Soleimani *et al.* 1987). In corneal endothelial cells a coupling ratio of 2:1 or 3:1 was compatible with the results (Jentsch *et al.* 1984). In the present study a stoichiometry of 2:1 for the inwardly directed co-transport in leech glial cells is suggested according to the thermodynamics of the system.

Being outwardly directed at physiological conditions, stimulation of the Na^+–HCO_3^- co-transport produced a membrane depolarization in epithelial cells. In both vertebrate epithelial cells and leech glial cells more HCO_3^- than Na^+ ions are carried by the Na^+–HCO_3^- co-transporter. Due to the opposite direction of the Na^+–HCO_3^- co-transporter in the different preparations this would lead to membrane depolarization or hyperpolarization, respectively.

Functional significance of a glial Na^+–HCO_3^- co-transport

The intracellular alkalinization which brings the H^+ equilibrium potential to less negative values, and the membrane hyperpolarization induced by the introduction of a CO_2–HCO_3^--buffered saline increases the electrochemical H^+ gradient across the glial cell membrane. In contrast, the electrochemical H^+ gradient across the neuronal membrane is maintained or even slightly decreased (Deitmer & Schlue, 1987*a*). The pH_i increase of the glial cells is associated with the HCO_3^- uptake, which also increases the buffering power of the glial cytoplasm. If the concentration of CO_2 increases in the nervous tissue, as may be caused by an increased neuronal activity, the pH in glial cells would go alkaline due to stimulation of Na^+–HCO_3^- co-transport. Indeed, an alkalinization has recently been measured in rat astrocytes following evoked electrical activity of nerve cells (Chesler & Kraig, 1987). Hence, stimulation of Na^+–HCO_3^- co-transport into glial cells and the associated increase of glial buffering power may be important mechanisms for the H^+ regulation in the brain.

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