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Sulfate-chloride exchange transport in a glioma cell line

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Transport of SO_4^{2-} was studied in the glioma cell line LRM55 to determine whether it is mediated by the Cl^-/HCO_3^- exchanger or the K^+/Cl^- cotransporter previously described in these cells (Wolpaw, E.W. and Martin, D.L. (1984) Brain Res. 297, 317–327). $^{35}SO_4^{2-}$ influx was saturable with SO_4^{2-}. External SO_4^{2-} stimulated $^{35}SO_4^{2-}$ efflux, indicating an exchange mechanism. External Cl^- was a competitive inhibitor of $^{35}SO_4^{2-}$ influx. Internal Cl^- stimulated $^{35}SO_4^{2-}$ influx and external Cl^- stimulated $^{35}SO_4^{2-}$ efflux, indicating that Cl^- is an exchange substrate for the SO_4^{2-} carrier. Also, SO_4^{2-} flux was sensitive to SITS, DIDS and furosemide. However, saturating external SO_4^{2-} did not inhibit $^{36}Cl^-$ influx and did not inhibit $^{36}Cl^-$ efflux via the Cl^-/HCO_3^- exchanger. Moreover, K^+ did not stimulate $^{35}SO_4^{2-}$ influx as it does Cl^- influx. These findings indicate that SO_4^{2-} transport into these cells is mediated by an exchange carrier distinct from both the Cl^-/HCO_3^- exchanger and the K^+/Cl^- cotransporter. While Cl^- is an alternative substrate for the SO_4^{2-} porter, this carrier is responsible for only a minor fraction of total Cl^- flux in these cells.

Introduction

Anion transport across the astroglial cell membrane appears to be an important mechanism for maintenance of the extracellular environment in the central nervous system [1,2]. Two major electroneutral anion transport systems are present in the astroglial cell membrane: a cation/anion cotransporter for K^+ and Cl^-, and an anion exchanger for Cl^- and HCO_3^- [3–5]. Cl^- transport systems are also present in many other cells. Of these, the erythrocyte anion exchanger is the most fully characterized. Under physiologic conditions it transports Cl^- and HCO_3^- and in vitro it also carries out homo- and hetero-exchange of SO_4^{2-} (reviewed in Refs. 6 and 7). Ehrlich ascites tumor cells also exchange SO_4^{2-} and Cl^- and may have additional Cl^- porters that do not carry SO_4^{2-} [8,9].

In the work presented here we sought to determine whether SO_4^{2-} transport in the glioma cell line LRM55 is mediated by either of the astrocytic Cl^- transporters previously described in these cells [5]. Our results show that SO_4^{2-} transport in LRM55 cells is carrier-mediated and that it occurs by an exchange mechanism. Although the SO_4^{2-} porter is able to transport Cl^- and is sensitive to compounds that inhibit Cl^- transport, our kinetic data indicate that this SO_4^{2-} porter is not the same as the major Cl^-/HCO_3^- exchanger or the K^+/Cl^- cotransporter previously described [5]. Rather, our findings indicate that the SO_4^{2-} porter in LRM55 cells is a separate carrier altogether.

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Abbreviations: DIDS, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SITS, 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid.

Materials and Methods

Methods

The methods used in this study were based on those previously described in detail [5,10].

Cell culture. LRM55 cells were originally cloned from a mixed glioma isolated from the spinal cord of a Fisher rat [10,11]. The cells were maintained in 100-mm diameter culture dishes and were seeded in 24-well culture dishes 6–9 days prior to each experiment. All experiments were carried out in 24-well dishes with cells from passages 6 to 26. At the time of the experiments, each well contained an average of 109 μg protein.

Flux assays. After preincubation of the cells in Hepes-buffered Hanks' balanced salt solution (HBHS) at 37°C for at least 20 min, uptake was initiated by replacing the HBHS with 200 μl of an osmotically equivalent salt solution containing the radioactively labeled uptake substrate. Uptake was terminated by rinsing the wells three times with 1 ml of HBHS at room temperature. All rinsing in these assays was performed using a syringe pipettor which was modified to direct the rinse solution evenly around the walls of the well and which incorporated an aspirator tube to remove the rinse solution immediately. By this method, cells could be rinsed three times in 7–10 s. Since over 95% of the extracellular fluid was removed in the first rinse and since the time required for all three rinses was very short compared to the total flux time, error due to uptake during the rinse procedure was negligible. To convert measured cpm to mol of influx substrate, we assumed that the specific activity of the labeled substrate in the medium did not change appreciably over the course of the experiment. This assumption was justified since the internal pool of SO_4^{2-} or Cl^- was no more than 0.2% of the total SO_4^{2-} or Cl^- in the experimental well.

In efflux experiments, cells were loaded with the labeled substrate by incubation in 200 μl of the labeled compound in HBHS. After a loading period of at least 30 min, the labeled medium was replaced with 800 μl or 1 ml of the appropriate efflux medium containing no label. Efflux was terminated by drawing off the medium and rapidly rinsing the well three times. Efflux was calculated as the percentage of the total label which remained in the cells at given time points after changing to unlabeled medium. Efflux was plotted as a function of time on semilogarithmic axes.

Correction for residual extracellular tracer. Since flux measurements were always made by assaying the cellular content of radiolabeled compounds, it was necessary to correct for residual label in the extracellular space. Extracellular residual label was measured in separate wells by removing HBHS, adding 200 μl of labeled incubation medium, and immediately rinsing three times. This amount was then subtracted from the total measured label for each flux data point. This method of measuring label in the residual extracellular fluid gave results similar to those obtained using impermeant molecules such as labeled sucrose and mannitol.

Scintillation counting and protein analysis. The cellular contents of the wells were dissolved and transferred to scintillation vials, radioactivity was measured by liquid scintillation and cellular protein was measured as previously described [5].

Solutions. The HBHS used in these experiments comprised 10 mM Hepes/11 mM glucose/130 mM Na^+/4.5 mM K^+/136 mM Cl^-/1.1 mM Ca^{2+}/0.71 mM Mg^{2+}/0.21 mM SO_4^{2-}/1.4 mM phosphate, and was adjusted to pH 7.3 with NaOH. Low-Cl^- media were based on this composition, with isosmotic replacement of NaCl by sucrose. For Cl^--free media, gluconate replaced the balance of the Cl^-. All drug-containing solutions were made up fresh each day. SITS and DIDS solutions were kept in opaque vessels and used in dim light.

In all experiments, the contributions of $^{35}SO_4^{2-}$ and $^{36}Cl^-$ to the total concentrations of SO_4^{2-} and Cl^- were considered in computing concentrations of these species.

Materials

The following isotopes were obtained from New England Nuclear, Boston, MA: $Na_2^{35}SO_4$ (in water, 10–1000 mCi/mmol), $^{36}Cl^-$ (as HCl, 6.1–6.4 mCi/g), and L-[4,5-^{3}H]leucine (58 Ci/mmol). SITS and DIDS were obtained from Pierce Chemical Company, Rockford, IL. Furosemide (5-[aminosulfonyl]-4-chloro-2-[(furanylmethyl)-amino]benzoic acid) was a gift from Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ.

Results

Sulfate influx

Since early experiments had shown that external Cl^- inhibited SO_4^{2-} influx, subsequent measurements of SO_4^{2-} influx were made in low-Cl^- or Cl^--free media. The time-course of $^{35}SO_4^{2-}$ influx in 7 mM Cl^- medium over a period of 90 min is shown in Fig. 1. An initial rapid burst of uptake of the labeled substrate reached a maximum by 15 min. This was followed by a decrease to a level that remained constant from 30 to 90 min, indicating that the extracellular $^{35}SO_4^{2-}$ had equilibrated with the intracellular pool. The unusual shape of this curve can be accounted for by the relationship between Cl^- and SO_4^{2-} transport to be described below and explained in detail in Discussion.

Kinetics of sulfate transport

Initial velocities of SO_4^{2-} transport were measured in Cl^--free medium over a range of SO_4^{2-} concentrations from 0.2 to 5 mM. A typical experiment is shown in Fig. 2. Initial rates could be described by the simple Michaelis-Menten equation. Kinetic parameters were obtained by fitting this equation to the data by a computer program based on the method of Eisenthal and Cornish-Bowden [12]. The apparent K_m was 0.24 mM and the apparent V_{max} was 1.2 nmol/min per mg protein (means of two experiments). The V_{max} for SO_4^{2-} flux is much smaller than the V_{max} for total Cl^- flux (280 nmol/min per mg protein) in these cells [5].

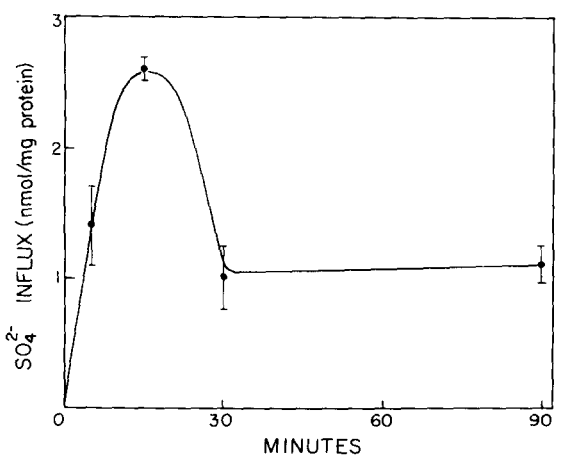

Fig. 1. Influx of $^{35}SO_4^{2-}$ at 37°C. Influx medium (low-Cl^- HBHS) contained all of the components of HBHS except that NaCl was reduced by equiosmolar replacement with sucrose so that $[Cl^-]$ was 7 mM and $[Na^+]$ was 9 mM. The concentration of SO_4^{2-} (including $^{35}SO_4^{2-}$) was 0.21 mM. Influx was initiated by replacing the preequilibrating HBHS with the labeled medium and was terminated by rinsing three times in HBHS. Each point is the mean of 3–6 determinations (± S.E.).

Steady-state sulfate content

The steady-state concentration of SO_4^{2-} in LRM55 cells was determined at 37°C after at least 30 min of uptake of $^{35}SO_4^{2-}$ from HBHS containing 0.21 mM SO_4^{2-}. Under these conditions, the cellular SO_4^{2-} content was 0.66 ± 0.03 nmol/mg protein (mean from nine experiments ± S.E.). Since the intracellular space of LRM55 cells is between 2.3 and 3.9 μl/mg protein [10,13], the internal concentration of SO_4^{2-} was between 0.17 and 0.29 mM. Thus, the internal and external concentrations of SO_4^{2-} were approximately equal.

Efflux of sulfate and homo-exchange

To explore the possibility that SO_4^{2-} transport occurred by an exchange mechanism, we first measured the efflux of $^{35}SO_4^{2-}$ into HBHS (Fig. 3A, lower curve). The initial rate of SO_4^{2-} efflux was 0.12 nmol/min per mg protein (mean from two experiments). After 10 min of incubation, only 40% of the label remained in the cells.

We then measured the efflux of $^{35}SO_4^{2-}$ in the presence and absence of 10 mM external SO_4^{2-}, with no other anions (except the Hepes buffer) included in the efflux medium (Fig. 3B). In the absence of external anions, $^{35}SO_4^{2-}$ efflux was slow, with only 8.5% of the label lost from the cells after 5 min of incubation. The presence of 10 mM external SO_4^{2-} caused a 2.8-fold increase in the initial rate of $^{35}SO_4^{2-}$ transport out of the cells. This trans-stimulation by external SO_4^{2-} suggests that SO_4^{2-} transport is mediated by an exchange porter which can carry $^{35}SO_4^{2-}$ from inside to outside in exchange for external SO_4^{2-}.

Effect of chloride on sulfate flux

The effect of Cl^- on SO_4^{2-} flux was also examined. As external $[Cl^-]$ was varied from 136 to 7.3 mM, SO_4^{2-} influx increased from 0.09 ± 0.002 to 0.43 ± 0.02 nmol/min per mg protein (means ± S.E.; $n = 3$). Measurements of SO_4^{2-} influx as a

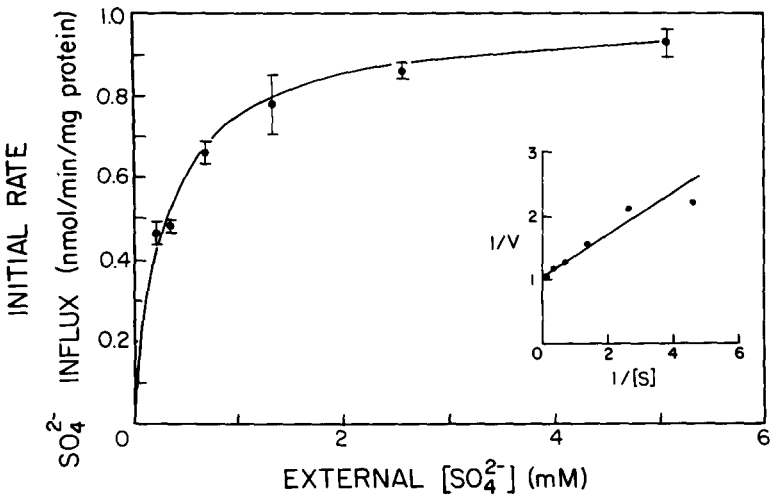

Fig. 2. Dependence of $^{35}SO_4^{2-}$ influx on SO_4^{2-} concentration. $[SO_4^{2-}]$ was varied by equimolar replacement with gluconate. Influx medium comprised 280 mM sucrose/10 mM Na^+/1.8 mM Mg^{2+}/10 mM Hepes, and the pH was adjusted to 7.3 with NaOH. Influx was initiated by replacing the preequilibrating HBHS with the labeled medium and was terminated at 2 min by rinsing three times with HBHS. Each data point is the mean of four determinations (± S.E.). Inset: double-reciprocal plot of the same data. The line was drawn using the slope and intercepts derived from the computer analysis of the data, according to the method of Eisenthal and Cornish-Bowden [12].

function of SO_4^{2-} concentration at three concentrations of Cl^- revealed that external Cl^- was a competitive inhibitor (Fig. 4). Computer analysis [14] of these data gave a K_i of 15.5 ± 1.4 mM (mean ± S.E.).

Since competitive inhibition by Cl^- suggested that Cl^- might be a substrate for the SO_4^{2-} exchanger, we examined the ability of Cl^- to trans-stimulate the efflux of SO_4^{2-} (Fig. 3C). External NaCl (140 mM) stimulated initial $^{35}SO_4^{2-}$ efflux by a factor of 3.3 over the control, which contained only sucrose and Hepes buffer. Thus, external Cl^- was able to trans-stimulate $^{35}SO_4^{2-}$ efflux and appeared to be more effective than SO_4^{2-} itself.

We also examined the ability of internal Cl^- to trans-stimulate SO_4^{2-} influx. Since incubation in low-Cl^- medium causes a decrease in steady-state

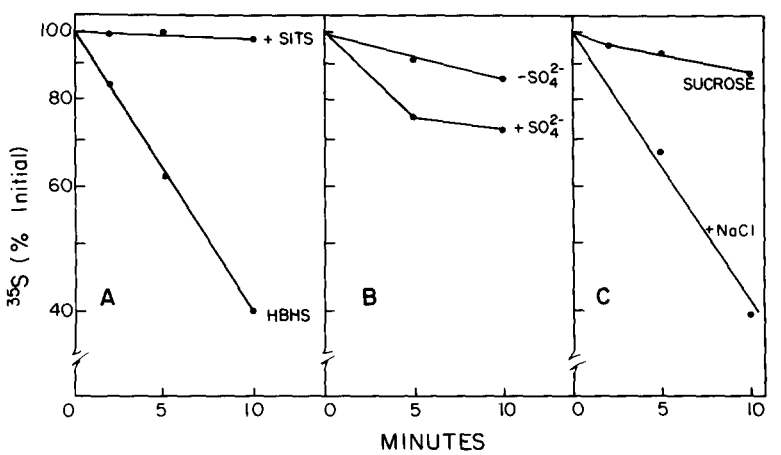

Fig. 3. Effect of SITS (A), external SO_4^{2-} (B) and external Cl^- (C) on efflux of $^{35}SO_4^{2-}$. Cells were loaded with $^{35}SO_4^{2-}$ by incubation for 30–70 min in HBHS containing $^{35}SO_4^{2-}$. Efflux was initiated by replacing the labeled medium with 800 or 1000 μl of the appropriate unlabeled medium and was terminated by rinsing three times with HBHS. Each point is the mean of four determinations. S.E. values are all less than 7% of the means. The ordinate is a logarithmic scale. (A) Effect of SITS. Efflux medium was HBHS, with or without 1 mM SITS. (B) Effect of external SO_4^{2-}. The SO_4^{2-}-containing efflux medium consisted of 10 mM SO_4^{2-}/20 mM Na^+/1 mM K^+/10 mM Hepes/270 mM sucrose. The SO_4^{2-}-free efflux medium comprised 1 mM K^+/10 mM Hepes/300 mM sucrose. (C) Effect of external Cl^-. The Cl^--containing efflux medium consisted of 140 mM Cl^-/140 mM Na^+/10 mM Hepes. The Cl^--free medium comprised 280 mM sucrose/10 mM Hepes. All media were adjusted to pH 7.3 with NaOH.

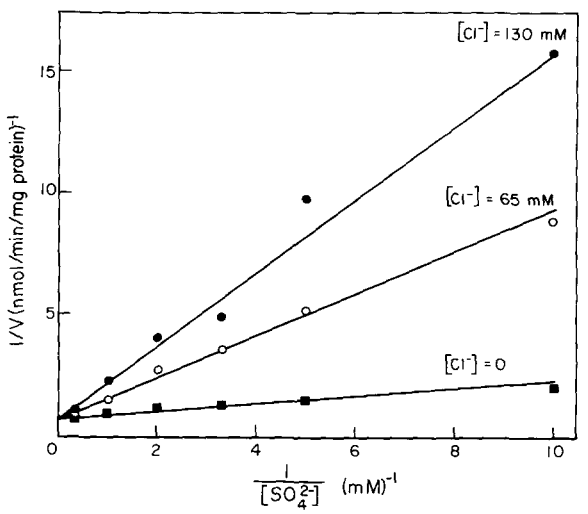

Fig. 4. Double-reciprocal plots of Cl^- inhibition of $^{35}SO_4^{2-}$ influx. The Cl^--free media comprised 4.1 mM K^+/0.71 mM Mg^{2+}/1.1 mM Ca^{2+}/11 mM glucose/7.7 mM gluconate/260 mM sucrose/10 mM Hepes. For the Cl^--containing media, NaCl was added by equiosmolar reduction of sucrose. The concentration of SO_4^{2-} was varied by equimolar replacement of SO_4^{2-} with gluconate. Influx was initiated by replacing the preequilibrating HBHS with the $^{35}SO_4^{2-}$-containing media and was terminated at 2 min by rinsing three times with HBHS. Each point is the mean of two or three determinations. The lines were drawn using the slopes and intercepts derived from computer analysis of the data, according to the method of Cleland [14].

Cl^- content of LRM55 cells [5], internal Cl^- was varied by preincubating the cells for 40 min in medium containing 7.3, 72, or 136 mM Cl^-. $^{35}SO_4^{2-}$ uptake was then measured in medium containing 0.21 mM SO_4^{2-} and 7.3 mM Cl^-. The initial rates of uptake into cells preincubated in 7.3 and 72 mM external Cl^- were 47 and 75% of that into the control cells preincubated in 136 mM Cl^- (data not shown). Thus, internal Cl^- was able to trans-stimulate $^{35}SO_4^{2-}$ influx.

Effect of chloride transport inhibitors on sulfate transport

Since our previous studies show that SITS, DIDS and furosemide inhibit Cl^- transport in LRM55 cells [5], we examined the effect of these compounds on SO_4^{2-} flux in these cells. SITS, a disulfonic acid stilbene derivative, was an effective inhibitor of SO_4^{2-} transport in LRM55 cells. At 1 mM, SITS inhibited SO_4^{2-} influx by 88% (Fig. 5A) and efflux by 94% (Fig. 3A). SO_4^{2-} influx was also inhibited 73% by 10 μM DIDS, another stilbene derivative (results not shown). The diuretic furosemide was also an effective inhibitor; at 5 mM, furosemide produced 98% inhibition of SO_4^{2-} influx (Fig. 5B).

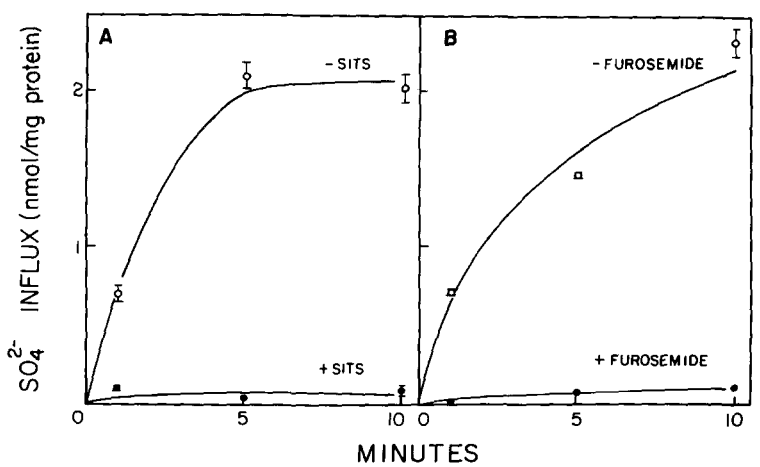

Fig. 5. Effect of SITS (A) and furosemide (B) on influx of $^{35}SO_4^{2-}$. Cells were preincubated for 30 min with (●) or without (○) the indicated inhibitor. Influx was initiated by replacing each preincubation medium with low-Cl^- medium containing $^{35}SO_4^{2-}$ and the inhibitor, and was terminated by rinsing three times with HBHS. Each point is the mean of three or four determinations (± S.E.) S.E. values are omitted where they are smaller than the symbols. (A) Effect of SITS. Influx medium was low-Cl^- HBHS (○) or low-Cl^- HBHS with 1 mM SITS (●). (B) Effect of furosemide. Influx media contained 18 mM Hepes, 19 mM glucose, 9.8 mM Na^+, 4.1 mM K^+, 6.6 mM Cl^-, 1.0 mM Ca^{2+}, 0.64 mM Mg^{2+}, 0.19 mM SO_4^{2-}, 1.2 mM phosphate, and either 5 mM furosemide (●) or 5 mM isethionate (○). All media were adjusted to pH 7.3 with NaOH.

Effect of sulfate on chloride flux

Since SO_4^{2-} exchange transport was competitively inhibited by Cl^-, since its pharmacologic sensitivities were similar to those of the glial Cl^-/HCO_3^- exchanger, and since Cl^- acted as a substrate for the SO_4^{2-} porter, it appeared initially that SO_4^{2-} transport was carried out by the Cl^-/HCO_3^- exchanger. If this hypothesis were correct, then external SO_4^{2-} ought to inhibit that portion of total Cl^- flux that is mediated by the Cl^-/HCO_3^- exchanger. To test for such inhibition, we held external [Cl^-] constant at 35 mM and measured the rate of $^{36}Cl^-$ uptake over a range of external SO_4^{2-} concentrations from 0.02 to 20 mM (Fig. 6). There was no significant inhibition of Cl^- influx at any concentration of SO_4^{2-}. Using the kinetic constants determined by the data shown in Fig. 4, we calculated that at 35 mM Cl^- and 20 mM SO_4^{2-}, the Cl^- exchanger would have been inhibited by 97% if SO_4^{2-} entered only on the major Cl^-/HCO_3^- exchanger. However, since Cl^- uptake via the Cl^-/HCO_3^- exchanger is only 63% of total Cl^- uptake in LRM55 cells [5], 20 mM SO_4^{2-} ought to have caused 61% inhibition of total Cl^- influx. This experiment was repeated under several different, but analogous conditions, with no evidence of any ability of SO_4^{2-} to inhibit $^{36}Cl^-$ uptake. The inability of SO_4^{2-} to inhibit Cl^- influx is not consistent with SO_4^{2-} transport by the Cl^-/HCO_3^- exchanger.

We performed another series of experiments to examine this question further. If the SO_4^{2-} porter were the same porter as the Cl^-/HCO_3^- exchanger as originally proposed, then consideration of the kinetic parameters for the two ions suggests that external SO_4^{2-} should be able to inhibit the transstimulation of Cl^- efflux by external Cl^-. That is, maximal SO_4^{2-} influx was only 1.2 nmol/min per

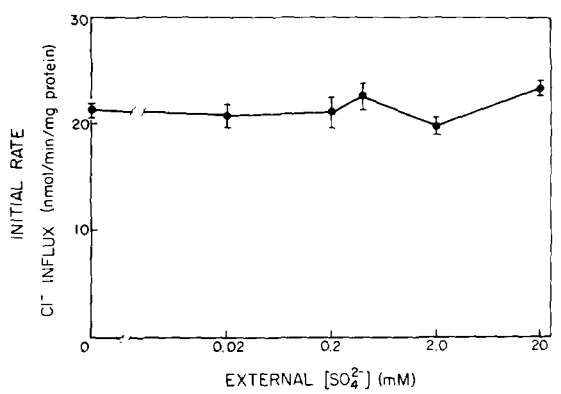

Fig. 6. Lack of effect of external SO_4^{2-} on influx of $^{36}Cl^-$. Influx was initiated by replacing the preequilibrating HBHS with influx media containing different concentrations of SO_4^{2-} and was terminated at 2 min by rinsing three times with HBHS. [SO_4^{2-}] was varied by the reciprocal equiosmolar variation of Na_2SO_4 and sucrose. All influx media also contained 35 mM Cl^-, 4.1 mM K^+, 0.7 mM Mg^{2+} and 10 mM Hepes, with sucrose comprising the balance (total osmolarity of 300 mM). Each point is the mean of four determinations (±S.E.).

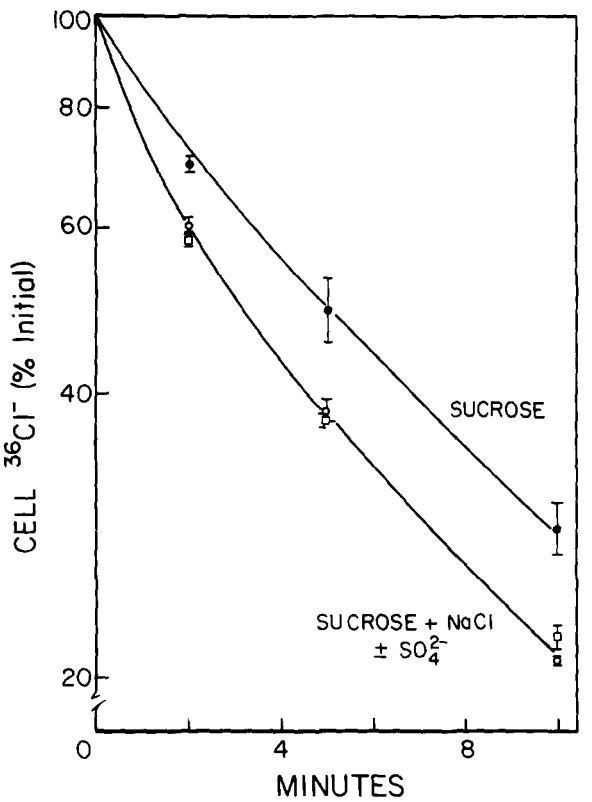

Fig. 7. Lack of inhibition of Cl^--stimulated $^{36}Cl^-$ efflux by external SO_4^{2-}. Cells were loaded with $^{36}Cl^-$ by incubation for 60 min in HBHS containing $^{36}Cl^-$. Efflux was initiated by replacing the labeled HBHS with one of three unlabeled efflux media and was terminated by rinsing three times in HBHS. The efflux media contained: (●) 260 mM sucrose and 3 mM gluconate; (○) 130 mM NaCl and 3 mM gluconate; or (□) 130 mM NaCl and 3 mM SO_4^{2-}. In addition, all of these media also contained 4.1 mM K^+, 1.1 mM Ca^{2+}, 0.71 mM Mg^{2+}, 8 mM gluconate, 11 mM glucose and 10 mM Hepes. Each point is the mean of four determinations (±S.E.). Control experiments not presented here showed that gluconate itself did not significantly affect Cl^- efflux.

mg protein, which is very slow relative to Cl^- flux [5]. Therefore, if the two porters were identical, saturating concentrations of external SO_4^{2-} would be expected to retard the stimulating effect of external Cl^-. To test this, we examined the effect of external SO_4^{2-} on the stimulation of $^{36}Cl^-$ efflux by external Cl^- (Fig. 7). In the absence of external SO_4^{2-}, external Cl^- alone (130 mM) stimulated efflux of $^{36}Cl^-$ from the cells as expected (compare open circles and closed circles, Fig. 7). The addition of 3 mM SO_4^{2-} to the 130 mM Cl^- medium (open squares, Fig. 7) caused no change in the stimulating effect of external Cl^-. At these concentrations of Cl^- and SO_4^{2-}, if SO_4^{2-} were a substrate for the Cl^-/HCO_3^- exchanger, SO_4^{2-} should have caused 56% inhibition of the Cl^--stimulated portion of Cl^- efflux. This was not the case. In other similar experiments, the concentration of SO_4^{2-} was raised as high as 20 mM but there was still no inhibition of the Cl^--stimulated efflux of $^{36}Cl^-$. Since SO_4^{2-} did not retard the stimulating effect of external Cl^-, SO_4^{2-} does not appear to be a substrate for the Cl^-/HCO_3^- exchanger.

Effect of potassium on sulfate flux

Like SO_4^{2-} transport, K^+/Cl^- cotransport in LRM55 cells is sensitive to furosemide [5]. It was thus important to determine whether SO_4^{2-} transport was mediated by the K^+/Cl^- cotransporter, although this seemed unlikely due to the inability of SO_4^{2-} to inhibit total Cl^- influx (Fig. 6). If SO_4^{2-} were cotransported with K^+, SO_4^{2-} influx should be dependent on external $[K^+]$. Therefore, we tested the effect of external $[K^+]$ on $^{35}SO_4^{2-}$ influx (Fig. 8A). SO_4^{2-} influx was the same whether external $[K^+]$ was 4.1 mM or 102 mM. This contrasted sharply with the strong effect of K^+ on Cl^- transport (Fig. 8B). These results suggest that the K^+/Cl^- porter is not a significant SO_4^{2-} porter.

Effects of other physiologic anions on sulfate influx

The results described above suggest that the SO_4^{2-} porter is a distinct porter which is able to carry Cl^- but is responsible for only a minor fraction of total Cl^- flux. It was thus of interest to determine whether there were other physiologically important substrates for this SO_4^{2-} carrier. We tested a series of organic anions for ability to inhibit SO_4^{2-} transport. These included nucleoside mono-, di- and triphosphates, cyclic AMP, glutamate, inorganic phosphate, pyridoxal phosphate and pyridoxamine phosphate. None of the compounds tested had any profound inhibitory effect on SO_4^{2-} influx (results not shown). Surprisingly, most of the test compounds stimulated rather than inhibited SO_4^{2-} influx. In contrast, the same compounds had no significant effect on Cl^- influx.

Discussion

Our results indicate that SO_4^{2-} transport in LRM55 cells occurs by an exchange mechanism. Although our kinetic data indicate that Cl^- is a substrate for the SO_4^{2-} exchanger, they also sug-

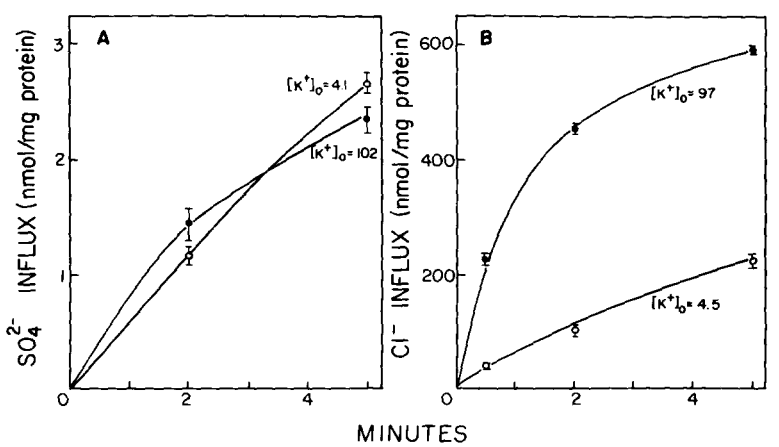

Fig. 8. Effect of external $[K^+]$ on influx of $^{35}SO_4^{2-}$ (A) and $^{36}Cl^-$ (B). Cells were preincubated for 20 min in HBHS with $[K^+]$ varied as indicated (in mM) by replacing Na^+ with K^+. For (A) $[Cl^-]$ was reduced to 7 mM by equiosmolar replacement of NaCl with sucrose. Influx was initiated by replacing each preincubation medium with medium containing $^{35}SO_4^{2-}$ or $^{36}Cl^-$ and was terminated by rinsing three times in ice-cold HBHS. Each point is the mean of three determinations (±S.E.); S.E. values are omitted where they are smaller than the symbols.

gest that the SO_4^{2-} transporter is not the same as the Cl^-/HCO_3^- exchanger or the K^+/Cl^- cotransporter previously described in these cells [5]. The SO_4^{2-} transporter appears, instead, to be a different porter which is able to carry Cl^- but is responsible for only a minor fraction of total Cl^- flux. This conclusion is based on the failure of SO_4^{2-} to inhibit Cl^- flux. If SO_4^{2-} were transported on either of the two major anion carriers, it would inhibit Cl^- influx. Moreover, if SO_4^{2-} were transported on the Cl^-/HCO_3^- exchanger, external SO_4^{2-} would inhibit Cl^--stimulated Cl^- efflux. However, we observed no such inhibition. In addition, K^+ did not stimulate SO_4^{2-} influx as it does Cl^- influx [5], providing further evidence that Cl^- does not enter by the K^+/Cl^- cotransporter.

The failure of SO_4^{2-} to inhibit Cl^- flux cannot be explained by application of Gunn's titratable carrier model [15] to the Cl^-/HCO_3^- exchanger in LRM55 cells. This model assumes that the Cl^-/HCO_3^- carrier exists in two interconvertible states which are titratable with H^+: a doubly charged state which binds divalent SO_4^{2-} and a singly charged state which binds monovalent Cl^- or HCO_3^-. However, since an equilibrium exists between these two states, the binding of SO_4^{2-} to the doubly charged state would shift the equilibrium in the direction of the doubly charged state. As the SO_4^{2-} concentration is raised, Cl^- transport would be inhibited due to the effective removal of the singly charged state of the carrier. Since our experiments show that SO_4^{2-} does not inhibit Cl^- transport in LRM55 cells, application of the model of Gunn to the Cl^-/HCO_3^- exchanger does not offer an explanation consistent with our results.

The kinetic data indicate that the SO_4^{2-} transporter in these cells can be saturated with SO_4^{2-}. The K_m for SO_4^{2-} (0.24 mM) is close to reported values for physiologic extracellular SO_4^{2-} [16]. Trans-stimulation of $^{35}SO_4^{2-}$ efflux by external SO_4^{2-} is strong evidence for an exchange mechanism. Moreover, since Cl^- is a competitive inhibitor and since it trans-stimulates SO_4^{2-} transport from either side of the cell membrane, Cl^- appears to act as an alternative substrate for the SO_4^{2-} porter. The biphasic time-course of $^{35}SO_4^{2-}$ influx is consistent with this interpretation. In the experiment shown in Fig. 1, influx was initiated by exposing the cells to medium containing $^{35}SO_4^{2-}$ and only 7 mM Cl^-. Since internal Cl^- is rapidly depleted when LRM55 cells are exposed to low-Cl^- medium [5], and since Cl^- and SO_4^{2-} appear to be mutually exchangeable substrates of the SO_4^{2-} porter, one would expect an initial phase of concentrative SO_4^{2-} uptake as external SO_4^{2-} exchanges for Cl^- leaving the cell. After internal Cl^- becomes depleted over the course of the first 15 min of SO_4^{2-} uptake, the accumulated $^{35}SO_4^{2-}$ exchanges with extracellular anions back across the membrane, reaching its steady-state level at 30 min.

The inhibitory effects of SITS, DIDS and furosemide suggest that the SO_4^{2-} porter may possess some structural similarity to the Cl^-/HCO_3^- exchanger and the K^+/Cl^- porter, as well as anion porters in other types of cells. However, these compounds are strong inhibitors not only of anion exchange but also of other Cl^--related processes in a wide variety of cell types [17–23]. Considering the wide reactivity of these compounds, it is not surprising that they inhibit the SO_4^{2-} exchanger along with other anion porters in LRM55 cells. It is noteworthy that these compounds are much more effective in inhibiting LRM55 SO_4^{2-} flux than Cl^- flux [5].

The issue of a membrane carrier that is able to exchange a divalent anion for a monovalent anion has been discussed by several authors. As noted above, Gunn [15] proposed that such a carrier may exist in two interconvertible forms which are titratable with H^+. Jennings' conclusion [24] that Cl^- exchange for SO_4^{2-} is accompanied by movement of one H^+ with each SO_4^{2-} supports Gunn's model in the erythrocyte anion exchanger. Callahan and Goldstein [25] suggested that carbonate crosses the human erythrocyte membrane as $NaCO_3^-$. By analogy, $NaSO_4^-$ may be the sulfate species that is transported by an anion porter which seeks a monovalent substrate for electroneutral exchange. A mechanism of SO_4^{2-} influx accompanied by Na^+ influx would unquestionably provide thermodynamic favorability for SO_4^{2-} uptake in our cells.

The physiologic role of the LRM55 SO_4^{2-} exchange porter is not clear. This porter does not appear to provide a major pathway for Cl^-. It does not appear to carry glutamate, inorganic phosphate, or any of a number of phosphorylated

small organic molecules. Moreover, the strong inhibitory effect of Cl^- at physiologic concentrations of Cl^- and SO_4^{2-} suggests that even SO_4^{2-} transport by this porter is largely inhibited under physiologic conditions. Assuming extracellular physiologic concentrations of 130 mM for Cl^- and 0.3 mM for SO_4^{2-} [16], the velocity of SO_4^{2-} uptake in LRM55 cells would be no greater than 13% of V_{max} for SO_4^{2-} under these conditions. However, even taking this Cl^- inhibition into account, SO_4^{2-} flux is quantitatively similar to the rates reported for astrocytic uptake of other compounds such as amino acids [10,23,26]. Moreover, quantitative cellular demand for SO_4^{2-} is probably not great in astrocytes. Sulfatides in the brain are found predominantly in myelin [27], although sulfatides and sulfated glycoproteins have also been identified in other brain membrane fractions [27,28]. Thus, LRM55 SO_4^{2-} flux, small though it is in comparison to Cl^- flux, is very likely sufficient to meet cellular metabolic demands.

SO_4^{2-} transport in LRM55 cells is in many respects similar to that in other types of cells. Like erythrocytes (reviewed in Refs. 6 and 7), LRM55 cells exchange SO_4^{2-} for Cl^-, but unlike erythrocytes, LRM55 cells do not transport SO_4^{2-} by the Cl^-/HCO_3^- exchanger. The similarities between anion transport in LRM55 cells and Ehrlich ascites tumor cells are very striking. In both cases, SO_4^{2-} is transported by homo-exchange or by hetero-exchange for Cl^- [8,9]. Also, SITS inhibits SO_4^{2-} flux almost completely but Cl^- flux only partially [5,9]. In both cases, Cl^- flux via the SO_4^{2-} pathway appears to account for only a minor fraction of total Cl^- flux [9,29]. Moreover, both types of cells appear to have two other mechanisms to account for the major portion of Cl^- flux: one carries out Cl^- self-exchange and does not transport SO_4^{2-}; the other carries out DIDS-insensitive K^+/Cl^- cotransport [5,9,30–34].

Membrane transporters like these have been described in cells from many other tissues and species including, among others, rabbit kidney and ileum [35–38], rat liver and small intestine [39,40], Madin-Darby canine kidney cells [21] and human and sheep erythrocytes [41,42]. Considering the differences in cell type and function among all of these cells, it is remarkable that they are so similar with respect to their anion transport properties. Many appear to have SO_4^{2-} exchange transporters which can also transport Cl^-. Many have K^+/Cl^- cotransporters and Cl^-/HCO_3^- exchangers. Although interest in each of these transporters initially focused on the specialized function of a particular type of cell, their widespread occurrence suggests that such mechanisms may not necessarily represent specialized functions of a particular cell type, but, like the ubiquitous $(Na^+ + K^+)$-ATPase, may be part of the general functional repertoire of mammalian cells.

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