

The Interaction of Intracellular Mg^{2+} and pH on Cl^{-} Fluxes Associated with Intracellular pH Regulation in Barnacle Muscle Fibers

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ABSTRACT The intracellular dialysis technique was used to measure unidirectional Cl^{-} fluxes and net acid extrusion by single muscle fibers from the giant barnacle. Decreasing pH_i below normal levels of 7.35 stimulated both Cl^{-} efflux and influx. These increases of Cl^{-} fluxes were blocked by disulfonic acid stilbene derivatives such as SITS and DIDS. The SITS-sensitive Cl^{-} efflux was sharply dependent upon pH_i , increasing ~ 20 -fold as pH_i was decreased from 7.35 to 6.7. Under conditions of normal intracellular Mg^{2+} concentration, the apparent pK_a for the activation of Cl^{-} efflux was 7.0. We found that raising $[Mg^{2+}]_i$, but not $[Mg^{2+}]_o$, had a pronounced inhibitory effect on both SITS-sensitive unidirectional Cl^{-} fluxes as well as on SITS-sensitive net acid extrusion. Increasing $[Mg^{2+}]_i$ shifted the apparent pK_a of Cl^{-} efflux to a more acid value without affecting the maximal flux that could be attained. This relation between pH_i and $[Mg^{2+}]_i$ on SITS-sensitive Cl^{-} efflux is consistent with a competition between H ions and Mg ions. We conclude that the SITS-inhibitable Cl^{-} fluxes are mediated by the pH_i -regulatory transport mechanism and that changes of intracellular Mg^{2+} levels can modify the activity of the pH_i regulator/anion transporter.

INTRODUCTION

Mg^{2+} is a key co-factor in the functioning of a variety of enzymes (Aikawa, 1981), especially for enzymes involved with transfer of phosphate groups (e.g., ATPases, phosphatases, kinases, etc.). However, high concentrations of Mg^{2+} have been shown to be inhibitory for some of the very processes for which this cation is a co-factor. For example, the Na pump or Na^{+},K^{+} -ATPase can be inhibited by intracellular Mg^{2+} concentrations ($[Mg^{2+}]_i$) in the range of 1–10 mM (e.g., De Weer, 1976; Flatman and Lew, 1981). Ca^{2+} efflux from squid axons is inhibited by raising the $[Mg^{2+}]_i$ (Requena, 1978). This effect is the result of partially inhibiting Na/Ca exchange without affecting the ATP-dependent Ca^{2+} efflux (DiPolo and Beaugé, 1985), both of which require Mg^{2+} for activation by ATP.

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Other possible inhibitory effects by Mg^{2+} might arise from competition with other cations for certain enzymes or intracellular receptors. Finally, internal Mg^{2+} may screen the fixed negative charges responsible for the surface potential at the membrane. Such charges might affect voltage-dependent functions that could be shifted if Mg^{2+} effectively reduced the surface potential. Surface charges can also affect the concentration of monovalent ions right at the surface of the membrane (McLaughlin et al., 1971).

Intracellular pH (pH_i) is maintained at a value more alkaline than equilibrium in a variety of cells by an Na-dependent Cl/HCO_3 exchange transport process (e.g., Russell and Boron, 1976; Thomas, 1976, 1977; Boron et al., 1979, 1981; Russell et al., 1983; L'Allemain et al., 1985). By means of this transport process, an intracellular Cl ion exchanges with an extracellular HCO_3 ion in an external Na-dependent manner to neutralize and/or eject two intracellular H ions (Boron and Russell, 1983). This transport process is inhibited by disulfonic acid stilbene derivatives such as SITS (4-acetamido-4'-isothiocyanostilbene disulfonic acid) and DIDS (4,4'-diisothiocyanostilbene disulfonic acid). Cyclic AMP stimulates this transporter in the barnacle muscle (Boron et al., 1978; Russell and Brodwick, 1981). In the squid giant axon, the Na-dependent Cl/HCO_3 transport mechanism has an absolute requirement for ATP (Russell and Boron, 1976; Boron and Russell, 1983).

We report here that reducing pH_i (in the nominal absence of HCO_3^-) causes a profound stimulation of the SITS-sensitive Cl^- efflux. We show that a steep, saturating dose-response relation exists between the SITS-sensitive Cl^- efflux and pH_i , as might be expected if this Cl^- efflux is mediated by the same mechanism, which, in the presence of HCO_3^- , engages in pH_i regulation or acid extrusion. We further report that increasing $[Mg^{2+}]_i$, but not $[Mg^{2+}]_o$, significantly reduces the SITS-sensitive Cl^- efflux and influx and acid extrusion. However, the potency of intracellular Mg^{2+} as an inhibitor is dependent upon pH_i , being greater the higher the pH_i . These data are consistent with a competition between H^+ and Mg^{2+} . Raising $[Ca^{2+}]_i$ had very minor effects on SITS-sensitive Cl^- fluxes.

Some of these results have been presented at the 28th Annual Biophysical Society Meeting (Russell et al., 1985).

MATERIALS AND METHODS

Giant barnacles were obtained either from Pacific Biomarine (Venice, CA) or Biomarine Enterprises (Seattle, WA). The animals used in these experiments were maintained in a seawater aquarium at 11°C for no more than 3 mo. Only fibers from the rostral and lateral scutal groups were used. Individual muscle fibers were separated from one another and the shell was split to produce several small groups of fibers still attached to the shell. A group was soaked in Ca^{2+} -free barnacle seawater (see below) for 30–40 min before cutting individual fibers from the shell. Individual fibers (900–1,500 μm in diameter, 2–4 cm in length) were then cannulated at both ends using glass cannulae ($\sim 1,000$ μm o.d., 800 μm i.d.) mounted horizontally in a specially designed internal dialysis chamber (Russell and Blaustein, 1975).

Solutions

The normal external fluid bathing the muscle fibers is denoted as BSW and had the following composition (millimoles per liter): 453 Na, 10 K, 11 Ca, 32 Mg, 544 Cl, 10 EPPS [(*N*-2-hydroxyethyl)-piperazine-*N'*-3-propanesulfonic acid], pH 8.0; osmolality, 960 mosmol/kg. When Mg was removed from the BSW (0-Mg BSW), it was replaced iso-osmotically with Na. When Ca was removed from the BSW (Ca-free BSW), it was replaced by Mg.

The intracellular dialysis fluid had the following composition (millimoles per liter): 180 K, 24.4 Na, 0–80 Mg, 30 Cl, 50.4–210.4 glutamate, 100 PIPES (piperazine-*N,N'*-bis-2-ethane-sulfonic acid; $pK_a = 6.8$), 400–574 mannitol, 5 EGTA, 0.5 phenol red, 4 ATP, pH 6.45–7.15; osmolality 1,010 mosmol/kg.

Isotope Techniques

Chlorine-36 was purchased from New England Nuclear (Boston, MA) as $H^{36}Cl$. It was neutralized with KOH or NaOH, gently evaporated to dryness, and redissolved in glass-distilled water to give a final $[Cl^-]$ of ~ 500 mM (actual $[Cl^-]$ measured using a chloridometer). Aliquots of this ^{36}Cl stock solution were added to the dialysis fluids or seawaters to yield a specific activity of ~ 0.03 – $0.1 \mu Ci/\mu mol$.

pH_i Measurements

The pH_i -sensitive microelectrodes and the recording techniques have been previously described (Russell et al., 1983). The more permeable dialysis tubing used in the present experiments (see below) gave better control of pH_i than was possible with the dialysis tubing previously used (cf. Russell et al., 1983).

Internal Dialysis

The original internal dialysis technique used very permeable glass tubes for controlling the intracellular solute composition (Brinley and Mullins, 1967). Such glass tubing has not been available from Corning for about 10 years. Since then, cellulose acetate tubing (FRL, Inc., Dedham, MA), rendered porous over the central region by hydrolysis in a mild alkaline solution, has been used. After hydrolysis, these dialysis tubes had an effective molecular-weight cut-off of $\sim 1,000$ daltons. We have previously noted that, in the barnacle muscle, the tubes did not permit very close control of pH_i ; at pH_i values significantly more acidic than normal (Russell et al., 1983).

Recently, Horn (1986) has developed a modification using a much more permeable dialysis tubing. For the present study, we have used a dialysis tubing with a nominal molecular-weight cut-off of 6,000 (Spectrapor HF MWCO 6000, Spectrum, Los Angeles, CA). The permeability of this tubing to ^{36}Cl , ^{45}Ca , and $[^{14}C]ATP$ is about threefold greater than that of the old-style tubing (Matsumoto, P., and J. M. Russell, manuscript submitted for publication). As can be seen in Fig. 1, control over pH_i , while not perfect, was quite good with the new, more permeable tubing. In addition, the dialysis tubing (250 μm o.d.) can sustain a unidirectional efflux of Cl^- of $\sim 12,000$ pmol/cm²·s ($[Cl^-] = 30$ mM). Thus, for a muscle fiber of 1,250 μm in diameter, the dialysis tubing could support a transsarcolemmal efflux of $12,000 \times (250/1,250) = 2,400$ pmol/cm²·s. This is almost three times the magnitude of the largest measured Cl^- flux in the present study. Therefore, we believe that the dialysis tubing is not rate-limiting for our measurements of unidirectional Cl^- fluxes.

Briefly, these new-style dialysis tubes consisted of a 12–14-cm length of the Spectrapor

tubing glued into a plastic T-tube. Dialysis fluid was pumped from a syringe via a length of PE-50 tubing (Clay-Adams, Parsippany, NJ) through a plastic T-tube, then into the Spectrapor tubing. The initial 3.5 cm of the Spectrapor tubing was covered with a thin coat of fingernail polish diluted 10–20 times with butyl acetate or acetone in order to limit permeability to the portion of the dialysis tube in the muscle fiber. A 100- μm -diam tungsten wire was inserted down the dialysis tube to make it stiff enough to be inserted longitudinally through the muscle fiber (see Fig. 2 in Boron and Russell, 1983, for a schematic of the dialysis set-up). After the positioning of the dialysis tube within the muscle fiber, the tungsten wire was removed and dialysis fluid flow was begun.

Determination of Free $[\text{Mg}^{2+}]$ in Dialysis Fluids

The dialysis fluids used in this study contained several ligands that can reduce the $[\text{Mg}^{2+}]$ of the final solution. These include ATP, EGTA, glutamate, and PIPES. Binding constants for Mg^{2+} with the latter three ligands, under the conditions of the present experiments, are not available in the literature. Therefore, we used Mg^{2+} -selective liquid ion-exchanger electrodes to determine the free $[\text{Mg}^{2+}]$ in our dialysis fluids. Macroelectrodes were fabricated fresh daily using fiber-filled capillary tubes (Ultrawick, World Precision Instruments, Inc., New Haven, CT) ~ 3 cm long. These tubes were silanized using trimethylchlorosilane vapors (Pfaltz & Bauer, Inc., Stamford, CT) at 250°C for 1 h. After cooling, the capillary was dipped into the Mg^{2+} -sensitive liquid exchanger (ETH 1117, Fluka Chemical Corp., Hauppauge, NY) for 5–7 min to permit capillary action to completely fill the electrode. The finished electrode was placed in a holder/connector filled with 100 mM KCl and 50 mM MgCl_2 . The reference electrode was an Ultrawick capillary filled with 100 mM KCl. Both the Mg^{2+} -sensitive and reference electrodes were connected to a high-impedance amplifier (model FD223, World Precision Instruments, Inc.) via calomel half-cells. Such electrodes, when calibrated in pure MgCl_2 solutions, had a slope of 25–27 mV/10-fold change in Mg^{2+} activity over the range 1–100 mM. These Mg^{2+} -sensitive macroelectrodes had a significant K^+ error (Lanter et al., 1980). Therefore, we routinely calibrated the electrodes using a variable $[\text{Mg}^{2+}]$ but a fixed $[\text{K}^+]$ of 180 mM to match the $[\text{K}^+]$ of the dialysis fluids used in this study. The pH of these calibrating solutions (pH_{DIF}) was 6.8 (1 mM PIPES). In this series of calibrating solutions, we found the electrode's slope to be 21–25 mV/10-fold change in Mg^{2+} activity in the 5–50 mM range of $[\text{Mg}^{2+}]$ and 16–20 mV over the 1–5 mM range.

We determined the apparent binding constants of glutamate, PIPES, and EGTA for Mg^{2+} using these electrodes. This was accomplished by measuring the $[\text{Mg}^{2+}]$ in solutions of constant total $[\text{Mg}]$ with either variable total [glutamate], variable total [PIPES], or variable total [EGTA]. For glutamate, 13 separate determinations yielded an apparent dissociation constant of $8.5 \pm 3.6 \times 10^{-2}$ M. For PIPES, five separate determinations yielded an apparent dissociation constant for Mg^{2+} -PIPES of $1.14 \pm 0.15 \times 10^{-1}$ M. For EGTA, three separate determinations yielded an apparent dissociation constant of $6.65 \pm 1.2 \times 10^{-2}$ M (cf. 8.09×10^{-2} M, at pH 7.1, from Caldwell, 1970).

Using these values and values in the literature for ATP-Mg binding (De Weer and Lowe, 1973), we calculated the expected $[\text{Mg}^{2+}]$ in our dialysis fluids. In addition, for two dialysis fluids, we directly measured the $[\text{Mg}^{2+}]$ with the Mg^{2+} -sensitive electrodes and compared these results with those expected from the calculation. For a dialysis fluid containing 44 mM total $[\text{Mg}]$, the calculated $[\text{Mg}^{2+}]$ was 12.6 mM, while that measured using the Mg^{2+} -sensitive macroelectrode was 16.0 mM. A second dialysis fluid containing 14 mM total $[\text{Mg}]$ had a calculated free $[\text{Mg}^{2+}]$ of 3.85 mM, while that measured with the macroelectrode was 4.9 mM. Although this degree of agreement is gratifying, we will

report not only the apparent $[Mg^{2+}]$ based on our calculations, but also the total Mg concentration ($[Mg]_i$) in each experimental condition.

RESULTS

Effects of Acidic pH_i on Cl^- Efflux

In the present work, we have used the new, more permeable, dialysis tubes to explore the relation between Cl^- efflux and pH_i . Although the external fluids were nominally HCO_3^- -free, equilibration with room air ($P_{CO_2} = 0.03\%$) at a pH_o of 8.0 results in an $[HCO_3^-]_o$ of ~ 0.8 mM. Since the $k_{0.5}$ of the pH_i regu-

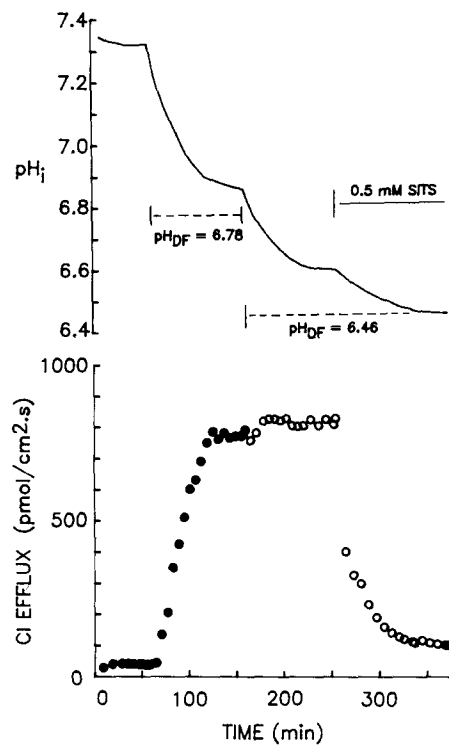


FIGURE 1. Effect of changing the pH of the dialysis fluid on Cl^- efflux and pH_i . Note that whereas decreasing pH_i from 7.31 to ~ 6.85 had a large effect on Cl^- efflux, further decreasing pH_i to ~ 6.6 had little additional effect. Also note that nearly all the acidic pH_i -stimulated Cl^- efflux was blocked by SITS and that application of SITS also resulted in the directly measured pH_i becoming equal to the pH of the dialysis fluid. Temperature, $20^\circ C$; fiber diameter, $1,175 \mu m$.

lator for $[HCO_3^-]_o$ has been reported to be ~ 3 mM (Boron et al., 1981), 0.8 mM is a sufficient $[HCO_3^-]_o$ to partially activate the acid-extrusion process. This partial activation of the pH_i regulator probably accounts for the differences noted between pH_{DF} and the directly measured pH_i in this study.

Fig. 1 shows the results from a single muscle fiber dialyzed with a solution containing normal concentrations of ATP (4 mM) and total Mg (7 mM). The unidirectional Cl^- efflux averaged ~ 50 pmol/cm 2 ·s when $pH_i = 7.35$ (cf. Russell and Brodwick, 1979). Reduction of pH_i by dialysis with a fluid identical in all respects except by being buffered to a pH of 6.78 (PIPES replaced HEPES) resulted in a directly measured fall of pH_i to ~ 6.85 (Fig. 1, top). Such a change

in pH_i was accompanied by a large increase of the Cl^- efflux (Fig. 1, bottom). In 12 fibers, the average Cl^- efflux at $\text{pH}_i = 6.85$ was $790 \pm 50 \text{ pmol/cm}^2 \cdot \text{s}$ (mean \pm SEM). However, a further reduction of pH_i to $\text{pH} 6.60$ caused little further increase in Cl^- efflux ($\bar{X} = 847 \pm 47$, $n = 7$). Treatment of acid pH_i -stimulated muscle fibers with SITS (0.2–0.5 mM) reduced Cl^- efflux to levels very near the flux observed before intracellular acidification ($\bar{X} = 107 \pm 55 \text{ pmol/cm}^2 \cdot \text{s}$, $n = 7$). Thus, Cl^- efflux, as well as influx (see below and Boron et al., 1978), are stimulated by acidic pH_i and this stimulation is SITS inhibitable.

The relationship between pH_i and the SITS-sensitive Cl^- efflux was explored in 31 fibers by varying pH_i between 7.4 and ~ 6.4 . As in the experiment illustrated in Fig. 1, pH_i was measured directly in every fiber. For each fiber, no more than two different pH_i values were tested. The collated results in Fig. 2 show that reducing pH_i from 7.35 to 6.8 resulted in an increase of the SITS-sensitive Cl^- efflux from near zero to $\sim 700 \text{ pmol/cm}^2 \cdot \text{s}$. Thus, an increase in

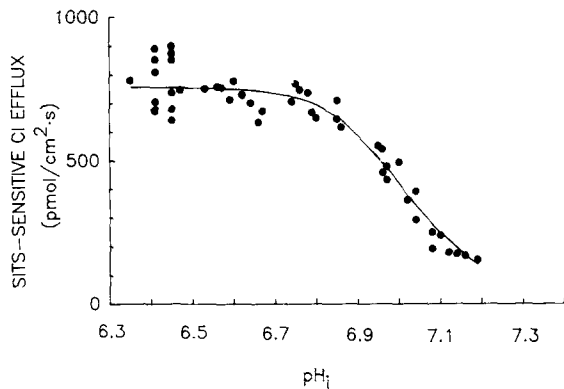


FIGURE 2. SITS-inhibited Cl^- efflux as a function of the directly measured pH_i . Single muscle fibers were dialyzed to various steady state pH_i values (measured with pH microelectrodes) and then 0.2 mM SITS was applied in the external fluid. Each point represents the difference between the Cl^- efflux in the absence and presence of SITS for a single fiber. No more than two pH_i val-

ues were tested in one fiber. The solid curve represents a best fit of the data by the Hill equation: $V_{\text{max}} = 750 \text{ pmol/cm}^2 \cdot \text{s}$, $k_{0.5} = 1.0 \times 10^{-7} \text{ M}$ ($\text{pH}_i = 7.0$); Hill coefficient = 5.1. This figure contains collated data from 31 muscle fibers. Temperature 20°C .

$[\text{H}^+]_i$ of ~ 3.5 times (4.5 to $15.8 \times 10^{-8} \text{ M}$) resulted in a 17.5-fold increase of the SITS-inhibitable Cl^- efflux. The half-saturation pH_i is ~ 7.0 . Finally, when Cl^- efflux was plotted as a function of $[\text{H}^+]_i$, a higher-order dependence upon $[\text{H}^+]_i$ was noted. These data were fitted best using the Hill equation having a Hill coefficient of between 3 and 5.

Effect of Varying $[\text{Mg}^{2+}]_o$ on the Acid-stimulated Cl^- Efflux

The control external fluid (BSW) contained 32 mM Mg. We examined the effects of increasing and decreasing the $[\text{Mg}^{2+}]_o$ on the acid-stimulated Cl^- efflux. Changes in the $[\text{Mg}^{2+}]_o$ were made by iso-osmotically varying $[\text{NaCl}]$ and $[\text{MgCl}_2]$. Thus, $[\text{Na}^+]_o$ varied from 383 to 496 mM, while $[\text{Cl}^-]_o$ varied from 544 to 588 mM. Such variations of these two ions are not believed to significantly affect the acid-stimulated efflux of Cl^- . Fig. 3 shows the effects of both increasing and decreasing $[\text{Mg}^{2+}]_o$ in a fiber dialyzed with a $\text{pH} 6.9$ fluid. Vary-

ing $[Mg^{2+}]_o$ between 0 and 100 mM had only a slight effect on the total Cl^- efflux. In five fibers, the Cl^- efflux when $[Mg^{2+}]_o$ was 32 mM (control) was 725 ± 48 pmol/cm²·s. When $[Mg^{2+}]_o$ was 0 mM, the efflux was 781 ± 56 pmol/cm²·s. When $[Mg^{2+}]_o$ was 100 mM, Cl^- efflux averaged 658 ± 26 pmol/cm²·s. These fibers all had normal responses to SITS, as seen in Fig. 3. Thus, a slight tendency for Cl^- efflux to decrease as $[Mg^{2+}]_o$ was increased was noted. Whether this result was a direct consequence of $[Mg^{2+}]_o$ or, as seems more likely, the result of a slight secondary increase of $[Mg^{2+}]_i$ (see below), is not clear. Thus, we conclude that changes of $[Mg^{2+}]_o$ have, at most, a very slight effect on Cl^- efflux.

Effect of Increased $[Mg^{2+}]_i$ on the Acid-stimulated Cl^- Efflux

$[Mg]_i$ in the standard dialysis fluids used in these studies was 7 mM, giving a calculated $[Mg^{2+}]_i$ in the dialysis fluid of 1.14 mM. When $[Mg]_i$ (and therefore $[Mg^{2+}]_i$) was increased, two interesting effects were noted. They are illustrated

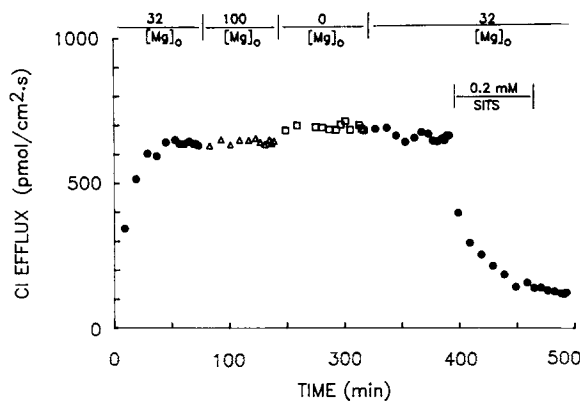


FIGURE 3. Experiment illustrating the small effect on acid pH_i -stimulated Cl^- efflux of varying $[Mg^{2+}]_o$. $pH_{DF} = 6.9$. As usual, SITS had a large inhibitory effect on Cl^- efflux. See text for details. Temperature, 20°C; fiber diameter, 1,245 μm .

in Fig. 4, which is representative of two other identical experiments. Under the initial conditions of $pH_{DF} = 6.78$, $[Mg]_i$ was 7 mM and the steady state Cl^- efflux was ~ 900 pmol/cm²·s, while the directly measured pH_i was ~ 6.85 . When $[Mg]_i$ was changed to 50 mM (estimated $[Mg^{2+}]_i = 17$ mM), both Cl^- efflux and pH_i were affected. Unidirectional Cl^- efflux decreased to ~ 300 pmol/cm²·s and pH_i became equal to pH_{DF} , 6.78. The effects of increasing $[Mg^{2+}]_i$ were readily reversible and $[Mg^{2+}]_i$ dependent, as can be seen when the dialysis fluid was changed to one containing an $[Mg]_i$ of 25 mM (estimated $[Mg^{2+}]_i = 9$ mM). This change resulted in a partial recovery of Cl^- efflux to a value of ~ 700 pmol/cm²·s. Furthermore, pH_i increased slightly, to ~ 6.81 . Finally, 0.5 mM SITS was applied externally and the Cl^- efflux decreased to ~ 100 pmol/cm²·s and pH_i decreased once again to 6.78.

Thus, the acid-stimulated Cl^- efflux was markedly sensitive to increases in the $[Mg]_i$ of the dialysis fluid. The SITS-sensitive Cl^- efflux at $pH_i = 6.8$ and $[Mg]_i = 7$ was ~ 800 pmol/cm²·s. Raising the $[Mg]_i$ to 50 mM inhibited Cl^- efflux by ~ 500 pmol/cm²·s, or $\sim 63\%$ of the total SITS-sensitive Cl^- efflux.

The effect on the directly measured pH_i implies that increasing $[\text{Mg}]_i$ also inhibited the acid-extruding mechanism. In Figs. 1 and 4, we see that treatment with SITS caused pH_i to relax to the same pH as the dialysis fluid. Presumably, this relaxation reflects the fact that SITS inhibits the acid-extruding mechanism (Boron et al., 1979, 1981). Similarly, we interpret the relaxation of pH_i to pH_{DF} after the increase of $[\text{Mg}]_i$ to mean that Mg^{2+} inhibits the activity of the acid-extruding mechanism (see below).

Increased $[\text{Mg}^{2+}]_i$ Inhibits Acid Extrusion

Evidence was presented above (see Fig. 4) that increasing $[\text{Mg}^{2+}]_i$ might inhibit the acid-extrusion mechanism of the barnacle muscle. Acid extrusion in barnacle

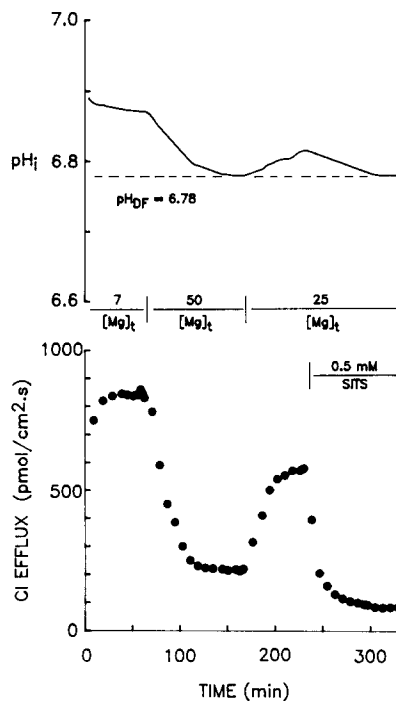


FIGURE 4. Experiment illustrating the effects of increased $[\text{Mg}^{2+}]_i$ on Cl^- efflux and on pH_i . 7 $[\text{Mg}]_i \cong 1.14 \text{ mM } [\text{Mg}^{2+}]_i$; 50 $[\text{Mg}]_i \cong 13.3 \text{ mM } [\text{Mg}^{2+}]_i$; 25 $[\text{Mg}]_i \cong 7.2 \text{ mM } [\text{Mg}^{2+}]_i$. Temperature, 20°C ; fiber diameter, $1,040 \mu\text{m}$.

muscle requires extracellular Na^+ and HCO_3^- (Boron et al., 1981), and is blocked by SITS (Boron, 1977; Russell et al., 1983). Evidence linking the acidic pH_i -stimulated Cl^- efflux to the operation of the acid-extruding mechanism would be provided by a demonstration that increased $[\text{Mg}^{2+}]_i$ inhibited recovery of pH_i from an acid load.

We tested this hypothesis directly in four fibers. The results of one such test are presented in Fig. 5. The pH_i was first reduced by dialyzing with a fluid lightly buffered with 10 mM MES at pH 6.45. The dialysis fluid contained 7 mM $[\text{Mg}]_i$ and 4 mM $[\text{ATP}]$. After reaching a directly measured pH_i of ~ 6.8 , the flow of dialysis fluid through the dialysis capillary was halted, which resulted in a slow increase of pH_i , presumably as a result of acid extrusion.

The top panel of Fig. 5 demonstrates the effect of superfusing the fiber with BSW containing 6 mM HCO_3^- ($P_{CO_2} = 0.4\%$; $pH = 8.0$). Under this set of conditions, the pH_i rapidly recovered toward the normal pH_i value of ~ 7.4 . Boron (1977) has reported the intrinsic buffering power of barnacle muscle to be 38.6 mM. By combining this intrinsic buffering power with the buffering capacity of 10 mM MES at $pH_i = 6.95$ (3.84 mM) and the contribution from the intracellular HCO_3^- generated from the CO_2 (2.25 mM), one can calculate the total buffer capacity (β) of these fibers to be ~ 45 mM. From this buffer capacity and the rate of change of pH_i at $pH_i = 6.95$, the net acid-extrusion rate was calculated to be $404 \text{ pmol/cm}^2 \cdot \text{s}$. After this treatment, the fiber was again dialyzed

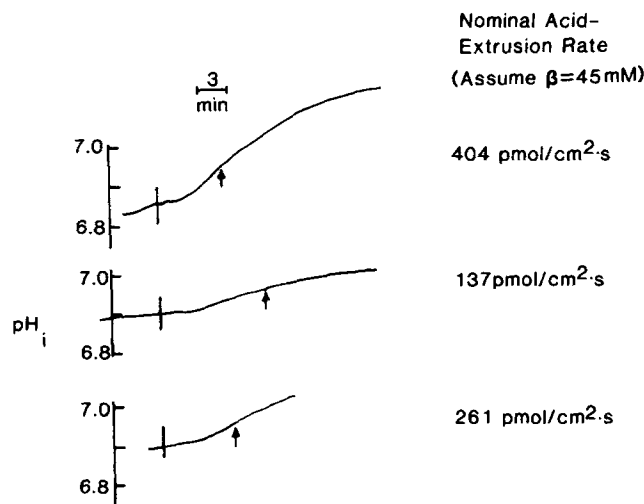


FIGURE 5. Effect of raising the $[Mg]_i$ on the acid-extrusion rate. Each trace represents the pH_i of a muscle fiber predialyzed with varying $[Mg]_i$. At the vertical mark, the muscle fiber was exposed to a seawater containing 6 mM HCO_3^- and 0.4% CO_2 . The arrow denotes $pH_i = 6.95$, at which point the slope of the dpH/dt function was measured. The acid-extrusion rate was calculated using dpH/dt and the total buffer capacity (β). From top to bottom: fiber equilibrated with 7, 25, 7 mM $[Mg]_i$. See text for details of this experiment. Temperature, 20°C ; fiber diameter, $1,190 \mu\text{m}$.

with a fluid identical to the first one, except that the $[Mg]_i$ was 25 mM. Dialysis was continued for 1 h to permit the sarcoplasmic $[Mg]_i$ to equilibrate with the new dialysis fluid $[Mg]_i$. In this case, when the 6 mM HCO_3^- BSW was applied after cessation of dialysis fluid flow, the pH_i recovered at a significantly lower rate, the nominal acid-extrusion flux being $\sim 140 \text{ pmol/cm}^2 \cdot \text{s}$. Finally, the same muscle fiber was again dialyzed for 1 h with the original dialysis fluid. When rechallenged with 6 mM HCO_3^- , the fiber responded with a nominal acid-extrusion rate of $\sim 260 \text{ pmol/cm}^2 \cdot \text{s}$. Although this rate was somewhat slower than the original rate (perhaps the $[Mg]_i$ had not returned to control levels), it still exceeded the rate measured when the fiber was equilibrated with a dialysis fluid

containing 25 mM $[Mg]_i$. In this fiber, the increased $[Mg]_i$ reduced the acid-extrusion rate from an average of 332 to 137 pmol/cm²·s.

In all four fibers treated according to this protocol, raising the $[Mg]_i$ reduced the acid-extrusion rate from an average of 226 ± 85 , at 7 mM $[Mg]_i$, to 82 ± 38 pmol/cm²·s, at 25 mM $[Mg]_i$, an inhibition of 63%. These results strongly support the hypothesis that increased $[Mg]_i$ inhibits the acid-stimulated Cl^- efflux by inhibiting the acid-extruding ion-transport mechanism that is the mediator of the acid-stimulated Cl^- efflux.

Relation Between pH_i and $[Mg^{2+}]_i$ -induced Inhibition of the Acid-stimulated Cl^- Efflux

In studies not shown, we found that $[Mg^{2+}]_i$ -induced inhibition of Cl^- efflux could be overcome by reducing pH_i . Thus, in order to quantitatively assess the relation between pH_i and the inhibition of the acid-stimulated Cl^- efflux by

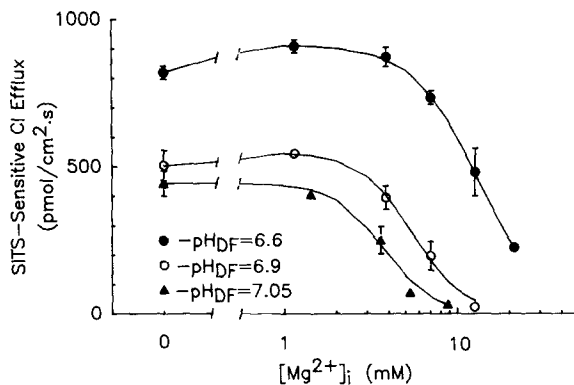


FIGURE 6. Relation between $[Mg^{2+}]_i$ and SITS-sensitive Cl^- efflux at three different pH_i values. Each point represents data averaged from at least three muscle fibers. A total of 61 muscle fibers were used in this study. Each fiber was dialyzed with a given pH_i and $[Mg^{2+}]_i$ for at least 3 h before treating with 0.2 mM SITS. $[Mg^{2+}]_i$ was calculated using the dissociation constants determined with the

Mg^{2+} -sensitive electrode (see Methods). The apparent inhibitory $k_{0.5}$ was 13.1 mM at $pH_{DF} = 6.6$, 5.5 mM at $pH_{DF} = 6.9$, and 3.8 mM at $pH_{DF} = 7.05$.

$[Mg^{2+}]_i$, a series of experiments was performed in which we varied the $[Mg]_i$ (at a constant $[ATP]$) at three different pH_i values. The general design of these experiments was to dialyze the fiber with a fluid at the required pH_i (6.6, 6.9, or 7.05), which contained 7 mM $[Mg]_i/4$ mM $[ATP]$. After a stable Cl^- efflux was obtained, the dialysis fluid was changed to a fluid containing the test $[Mg]_i/4$ mM $[ATP]$. After a stable efflux was achieved with this fluid, the fiber was superfused with BSW containing 0.2 mM SITS. No fewer than three fibers were tested at every $[Mg]_i$ for any given pH_i . A total of 61 fibers were tested. The collated results are seen in Fig. 6, plotted as the SITS-sensitive Cl^- efflux against the calculated $[Mg^{2+}]_i$. This figure clearly shows that raising the $[Mg^{2+}]_i$ inhibits the SITS-sensitive Cl^- efflux at every pH_i tested. However, the apparent inhibitory $k_{0.5}$ for Mg^{2+} was markedly affected by the pH_i . Mg^{2+} became a more potent inhibitor as pH_i became relatively more alkaline. Reducing $[Mg]_i$ to nominally 0 mM had minor effects, being slightly inhibitory at the most acidic pH_{DF}

tested (6.8), but slightly stimulatory at the most alkaline pH_{DF} tested (7.05).

The observation that raising the pH_i increased the apparent inhibitory potency of Mg^{2+} suggested that intracellular Mg^{2+} might be acting to shift the pH_i/Cl^- efflux relationship along the pH_i axis. We tested this hypothesis directly by measuring the SITS-inhibitable Cl^- efflux over a range of pH_i values while $[Mg]_i$ was maintained constant at 25 mM. Fig. 7 shows the collated results from 22 muscle fibers treated with 25 mM $[Mg]_i$ compared to the data already presented in Fig. 2 from fibers treated with 7 mM $[Mg]_i$. The effect of increasing $[Mg^{2+}]_i$ was to shift the apparent pK_a for the activation of Cl^- efflux from ~ 7.0 to 6.76 with little or no change in the maximal Cl^- efflux measured.

Another important observation can be made from these data by comparing them with the Mg^{2+} -induced inhibition of acid extrusion (see Fig. 5). The latter experiments were performed at a pH_i of 6.95. When one compares the degree

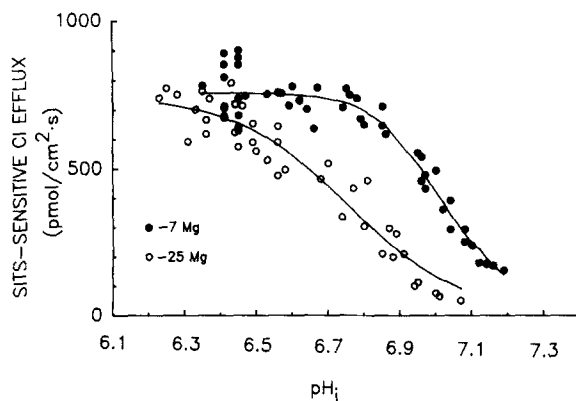


FIGURE 7. Comparison of H^+ activation of SITS-sensitive Cl^- efflux at two different $[Mg^{2+}]_i$ levels. The data for 7 mM $[Mg]_i$ are the same as in Fig. 2 replotted on an expanded pH_i scale. Data at 25 mM $[Mg]_i$ were obtained in the same way as described for 7 mM $[Mg]_i$. The solid curve through these latter data represents a best fit by the Hill equation with the following parameters: $V_{max} = 748 \text{ pmol/cm}^2 \cdot \text{s}$; $K_{0.5} = 1.75 \times 10^{-7} \text{ M}$ ($pH_i = 6.76$); Hill coefficient = 3.0. The data at 25 mM $[Mg]_i$ were obtained from 22 muscle fibers.

of inhibition by 25 mM $[Mg]_i/4 \text{ mM [ATP]}$ on acid extrusion with that of 24 mM $[Mg]_i/4 \text{ mM [ATP]}$ on Cl^- efflux at $pH_{DF} = 6.9$, one can see that both are inhibited by $\sim 63\%$. This provides further evidence that the SITS-sensitive, acid-stimulated Cl^- efflux is mediated by the same transport mechanism that mediates acid extrusion.

Effect of Varying $[Mg^{2+}]_i$ on Cl^- Influx

We have shown previously that the acid-extrusion process is reversible and hence ought to mediate Cl^- influx as well as efflux (Russell et al., 1983). We therefore examined the effect of raising the $[Mg]_i$ from 7 to 25 mM (estimated $[Mg^{2+}]_i = 1.14$ and 7.0 mM, respectively, at $pH_{DF} = 6.9$) on the SITS-sensitive Cl^- influx. Fig. 8 shows that just as was the case for efflux, the Cl^- influx was significantly inhibited by raising intracellular Mg^{2+} levels. A total of five fibers

were treated according to the protocol shown in Fig. 8. The average Cl^- influx when $[\text{Mg}]_i$ was 7 mM was 726 ± 49 $\text{pmol}/\text{cm}^2 \cdot \text{s}$. After increasing $[\text{Mg}]_i$ to 25 mM, the average Cl^- influx was 453 ± 9 $\text{pmol}/\text{cm}^2 \cdot \text{s}$. Treatment with 0.2 mM SITS reduced the Cl^- influx to 144 ± 18 $\text{pmol}/\text{cm}^2 \cdot \text{s}$. Thus, varying $[\text{Mg}^{2+}]_i$ from 1.14 to 7.2 mM resulted in a reduction of the SITS-sensitive Cl^- influx of 273 $\text{pmol}/\text{cm}^2 \cdot \text{s}$. This is close to the value of 210 $\text{pmol}/\text{cm}^2 \cdot \text{s}$ observed for the Mg-inhibited, SITS-sensitive Cl^- efflux over the same range of $[\text{Mg}^{2+}]_i$ (see Fig. 6, $\text{pH}_{\text{DF}} = 6.9$). These data support the notion that, in the nominal absence of external HCO_3^- , the anion transporter/ pH_i regulator engages in SITS-sensitive Cl/Cl exchange flux and that this flux is inhibited by increases of $[\text{Mg}^{2+}]_i$.

Thus, at $\text{pH}_{\text{DF}} = 6.9$ ($\text{pH}_i = 6.95$), the SITS-sensitive Cl^- influx was ~ 580 $\text{pmol}/\text{cm}^2 \cdot \text{s}$, while the SITS-sensitive Cl^- efflux under the same conditions was ~ 500 $\text{pmol}/\text{cm}^2 \cdot \text{s}$ (see Fig. 2). Given the scatter of the data and the technical differences between the influx and efflux determinations, these data are consistent with a 1:1 Cl/Cl exchanger. In both cases, 25 mM $[\text{Mg}]_i$ inhibited the flux

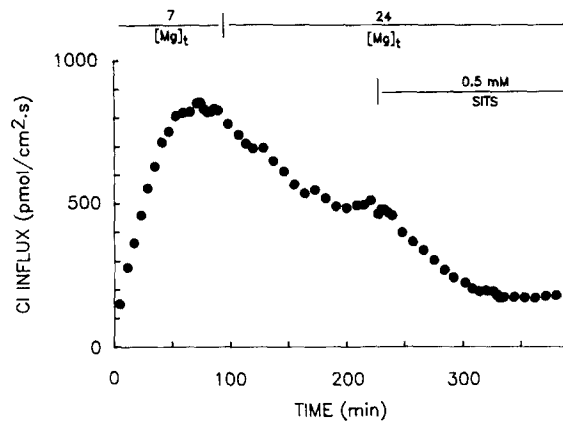


FIGURE 8. Effect of raising $[\text{Mg}]_i$ on Cl^- influx. Temperature, 20°C ; fiber diameter, $1,400 \mu\text{m}$.

by 200–250 $\text{pmol}/\text{cm}^2 \cdot \text{s}$, which further supports the notion that pH_i -stimulated, SITS-sensitive Cl^- fluxes are via an anion-exchange transporter.

Since in the presence of HCO_3^- a SITS-sensitive exchanger mediates Na-dependent Cl/HCO_3 exchange (acid-extrusion mode), one possibility is that the external HCO_3^- (plus Na) competes with external Cl^- so that, instead of Cl/Cl exchange, the transporter mediates Na-dependent Cl/HCO_3 exchange. Therefore, it is of interest to note that at $\text{pH}_i = 6.95$ and 7 mM $[\text{Mg}]_i$, in the presence of 6 mM HCO_3^- , the acid-extrusion rate was ~ 225 $\text{pmol}/\text{cm}^2 \cdot \text{s}$ (see above). If, under these conditions, the SITS-sensitive Cl^- efflux is the same as in the absence of HCO_3^- and if the stoichiometry for Cl^- efflux/net H^+ efflux is the same for barnacle muscle as for squid axon (i.e., 1 Cl^- :2 H^+), the acid-extrusion rate could, in principle, have been as high as $2 \times 500 = 1,000$ $\text{pmol}/\text{cm}^2 \cdot \text{s}$ instead of the 225 $\text{pmol}/\text{cm}^2 \cdot \text{s}$ actually measured. Although several assumptions were made in this analysis that must be directly tested, the analysis suggests that

not all the transporters engaged in Cl^-/Cl^- exchange are converted to Na-dependent Cl^-/HCO_3^- exchangers under the conditions of the present experiments. One possibility is that this difference reflects "reserve" anion transporters, which, under the appropriate conditions (increased $[HCO_3^-]$? decreased pH_i ?), can shift from the Cl^-/Cl^- exchange mode to the acid-extruding mode, i.e., Na-dependent Cl^-/HCO_3^- exchange. Alternatively, this result could mean that the turnover rate for transporters engaged in Na-dependent Cl^-/HCO_3^- exchange is less than for those transporters engaged in Cl^-/Cl^- exchange.

Effects of $[Ca^{2+}]_i$ on Cl^- Efflux

Ca^{2+} is normally present in barnacle sarcoplasm at ~ 100 nM (Hagiwara and Nakajima, 1966). The experiments reported to this point were conducted using dialysis fluids that were nominally Ca^{2+} -free and contained 5 mM EGTA. Analysis of these fluids revealed that they contained ~ 60 μM total Ca (as contami-

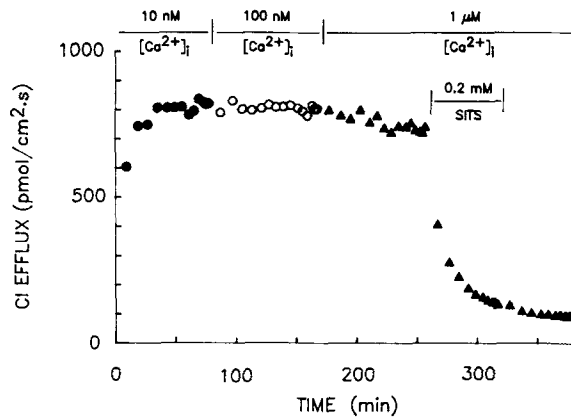


FIGURE 9. Effect of varying $[Ca^{2+}]_i$ on Cl^- efflux. Treatment with SITS revealed the same large SITS-sensitive Cl^- efflux seen in fibers dialyzed with very low $[Ca^{2+}]_i$. Temperature, $20^\circ C$; fiber diameter, $1,250$ μm .

nants of the known components of the fluids). Thus, the actual $[Ca^{2+}]_i$ of the dialysis fluids was ~ 10 nM. For the following experiments, we made no attempt to measure the actual levels of cytoplasmic Ca^{2+} , so the values reported are estimates of $[Ca^{2+}]_i$ in the dialysis fluid using a Ca/EGTA dissociation constant of 1.3×10^{-7} M (interpolated from Caldwell, 1970, at $pH = 7.0$). The total [EGTA] was always 5 mM.

Fig. 9 shows the effects of raising the nominal $[Ca^{2+}]_i$ stepwise from ~ 10 nM to 1 μM . The result in three fibers treated in an identical fashion was a slight but reproducible inhibition of Cl^- efflux as the nominal $[Ca^{2+}]_i$ was raised. As $[Ca^{2+}]_i$ was increased from 10 nM to 1 μM , the average Cl^- efflux decreased from 804 ± 28 to 679 ± 36 $pmol/cm^2 \cdot s$. Thus, increasing $[Ca^{2+}]_i$ in the physiological range had only small effects upon acid-stimulated Cl^- efflux. Unfortunately, raising $[Ca^{2+}]_i$ higher than 1 μM often resulted in an increase of Cl^- efflux that was not blocked by SITS and in progressive membrane potential depolarization.

DISCUSSION

Three central findings emerge from the results we have reported. First, the stimulation of SITS-inhibitable Cl^- efflux caused by a fall of pH_i has a very steep dependence upon $[\text{H}^+]_i$. Second, SITS-sensitive Cl^- fluxes (both efflux and influx), as well as net acid extrusion, are all inhibited by increases of $[\text{Mg}^{2+}]_i$. Third, the inhibition of Cl^- efflux caused by Mg^{2+} appears to be competitive with H^+ . In the following paragraphs, we will discuss these findings in terms of mechanisms and what they tell us about the mechanism of pH_i regulation and anion transport.

SITS-sensitive, pH_i -stimulated Cl^- Fluxes Are Probably via the pH_i -regulating Transporter

We have shown that the relationship between pH_i and Cl^- efflux is steep, having a Hill coefficient between 3 and 5 (see Figs. 2 and 7). The simplest interpretation of these results is that Cl^- efflux is stimulated by the simultaneous occupation by H ions of three to five sites. Such high Hill coefficients (n) for the effects of H^+ are not unusual, having been demonstrated for the closure of gap junction channels ($n = 4.5$; Spray and Bennett, 1985) and the inactivation of inward-rectifier channels of starfish eggs ($n = 3-4$; Moody and Hagiwara, 1982). Although no calculated Hill coefficients were presented, the steepness of the titration curves makes Hill coefficients >1 likely for titration of the Na-K pump in barnacle muscle (Russell et al., 1983), squid axon (Breitwieser et al., 1987), and urinary bladder (Eaton et al., 1984). Moreover, just as in the present case, the pK_a 's for all these effects were near 7.0. Thus, the pK_a and the steep H^+ dependence taken together imply that changes of pH_i in the physiological range may be a significant modulator of a number of important cellular processes.

Of particular interest to the present study is the observation that a fall of pH_i activates the transport systems that perform net acid extrusion (e.g., Roos and Boron, 1981). We have previously suggested that the transport mechanism in barnacle muscle that mediates the SITS-sensitive Cl^- transport is also responsible for pH_i regulation (Boron et al., 1978; Russell and Brodwick, 1981; Russell et al., 1983). Evidence in favor of this hypothesis includes: (a) a Cl^- requirement for the HCO_3^- -dependent and SITS-inhibitable Na^+ influx that occurs when pH_i is made acidic (Russell et al., 1983); (b) an extracellular Cl^- requirement for the SITS-sensitive reversal of acid extrusion (Russell et al., 1983); and (c) a cyclic AMP stimulation of acid extrusion and SITS-inhibitable Cl^- efflux (Boron et al., 1978). The present results showing a high degree of sensitivity of Cl^- efflux to $[\text{H}^+]_i$ (and the inhibition of acid extrusion and Cl^- fluxes by increased $[\text{Mg}^{2+}]_i$; see below) lend further support to this hypothesis.

Does the SITS-sensitive Cl^- Efflux Require a Co-Cation?

We have previously (Russell et al., 1983) postulated a number of hypothetical modes in which the pH_i -regulating transporter might operate. Four of these modes (modes 2, 9, 14, 15; Table V, Russell et al., 1983) would mediate Cl^-/Cl^- exchange as measured in the present experiments. However, three of the afore-

mentioned modes would mediate Na^+ fluxes as well. We have reported that in the nominal absence of HCO_3^- very little SITS-sensitive Na^+ influx or efflux can be detected (Russell et al., 1983), so it is unlikely that modes 2, 9, or 15 would be responsible for the present results. Further experiments will be required to determine whether the Cl^-/Cl^- exchange we discuss here is actually $H-Cl/H-Cl$ exchange.

The Nature of the pH_i -sensitive Site That Stimulates Cl^- Fluxes

The exact location of the pH_i -sensitive site or sites responsible for activation of acid extrusion and the associated Cl^- fluxes is uncertain. However, it is clear that the site (or sites) must be located on the internal face of the membrane since external acidification is much less effective in stimulating Cl^- efflux (Russell, J. M., and M. S. Brodwick, unpublished observations). In addition, Boron et al. (1979) have demonstrated that external acid actually inhibits the net acid-extrusion process. It is worth mentioning that the pH_i -sensitive sites(s) need not be located on the ion-transport molecule. Recently, we (Boron et al., 1988) have shown that the pH_i sensitivity of net acid extrusion in squid giant axon may be the result of pH_i sensitivity of a protein phosphatase that renders the transporter incapable of performing acid extrusion when it dephosphorylates the Na-dependent Cl^-/HCO_3^- exchanger. It appears that this protein phosphatase in the squid axon is inactivated at acidic pH_i values.

The pK_a of titration curves can be used to suggest the identity of the titrable chemical group. The apparent pK_a of 6.8–7.0 reported here for the internal sites responsible for activating the SITS-sensitive Cl^- efflux is consistent with several chemical moieties, including an imidazole group, a shifted carboxyl group, an alpha-amino acid group, and a sulfhydryl group. While our results are consistent with any of these groups, clear distinctions will require studies with group-specific reagents (see Means and Feeney, 1971).

The Interaction Between $[Mg^{2+}]_i$ and $[H^+]_i$

We have shown that raising $[Mg^{2+}]_i$ above 2–3 mM inhibits both the $[H^+]_i$ -stimulated Cl^- fluxes and acid extrusion. It is particularly interesting that this inhibition is a function of pH_i . Thus, the activation of Cl^- efflux caused by decreasing pH_i was shifted to the left (i.e., toward higher $[H^+]_i$ values) by increases of $[Mg^{2+}]_i$. This behavior is exactly what would be expected if H^+ and Mg^{2+} were competing for binding sites. Competition between H^+ and divalent cations in other biological systems is well known. For example, competition between Ca^{2+} and H^+ has been noted in the blockade of Na^+ channels of the node of Ranvier in frog nerve (Woodhull and Hille, 1970).

In addition to direct competition for common sites, divalent cations are capable of neutralizing the negative surface potential of the cell membrane (McLaughlin et al., 1971). Since the monovalent ion concentration right at the membrane surface is given by multiplying the bulk aqueous concentration of the monovalent ion by a Boltzmann factor whose exponent contains the surface potential, it follows that divalent cations can affect the surface concentrations of monovalents by neutralizing this surface potential. Such neutralization can

occur either by screening or by binding. For a screening mechanism, the effect of Mg^{2+} would not be specific; similar effects ought to be found with other divalent cations. Although we found no effect by Ca^{2+} comparable to that by Mg^{2+} , this result is inconclusive for the surface charge hypothesis because the highest $[Ca^{2+}]_i$ we could use before the muscle fiber became damaged was only $1 \mu M$, and screening of membrane surface charges requires millimolar concentrations of divalent cations. In preliminary experiments, we have found that Ba^{2+} also inhibits the pH_i -stimulated Cl^- efflux. However, Ba^{2+} precipitates ATP and thus the effect might be the result of lowering the $[ATP]_i$. More conclusive results regarding this hypothesis might be obtained by examining the effect of dimethonium on the pH_i -stimulated Cl^- efflux. Dimethonium has been reported to exert only screening effects and not to bind to divalent cation sites (McLaughlin et al., 1983).

Possible Sites of Mg^{2+}/H^+ Interaction

The site of the postulated Mg^{2+}/H^+ competition cannot be ascertained from the present results beyond saying that it must be an intracellular one. Changes of $[Mg^{2+}]_o$ had very little effect on Cl^- efflux and the small effect that did occur could very well be explained by changes of $[Mg^{2+}]_i$ occurring in the immediate subsarcolemmal space, where the distance from the dialysis capillary may reduce control by intracellular dialysis. Thus, both H^+ and Mg^{2+} exert their effects on the pH_i regulator/anion transporter at intracellular sites.

The involvement of Mg^{2+} with phosphate-transferring enzymes such as ATPases, protein kinases, and protein phosphatases leads to the suspicion that the effects reported here could be related to a phosphorylation/dephosphorylation mechanism. The Na-dependent Cl/HCO_3 exchanger in the squid giant axon has an absolute requirement for ATP (Russell and Boron, 1976; Boron and Russell, 1983) and recent evidence favors a protein kinase/protein phosphatase mechanism for activation/inactivation of this acid-extruding process (Boron et al., 1988). Thus, our evidence in the squid axon shows that the anion transporter/ pH_i regulator must be phosphorylated to be active. Our recent findings in the axon further suggest that the phosphatase responsible for the termination of transport activity is itself inactivated by a fall of pH_i . It is tempting to speculate that the inhibitory action of Mg^{2+} reported for barnacle muscle in the present work could be the result of Mg^{2+} preventing H ions from reaching inactivating sites on the protein phosphatase. This would result in the activation of a protein phosphatase, which would cause the dephosphorylation of the transporter, thereby inactivating the pH_i regulator/anion transporter. Alternatively, a protein kinase responsible for phosphorylating the transporter (or necessary precursor) could be inhibited by high $[Mg^{2+}]_i$. Inhibition of the activity of a cyclic AMP-dependent protein kinase by Mg^{2+} has been reported (Chiu and Tao, 1978). This effect has been suggested by Bittar and Chambers (1984) to explain the inhibitory effect of Mg^{2+} on a cyclic AMP-induced, ouabain-insensitive, Na^+ efflux in barnacle muscle. It should be noted that an ATP requirement by the pH_i regulator/anion transporter in snail neurons could not be demonstrated (Thomas, 1978, 1982), nor has it been directly demonstrated in

barnacle muscle. Efforts to do so in barnacle muscle have been thwarted by an inability to completely deplete the muscle fiber of ATP; levels of 20–50 μ M are the lowest so far achieved (Breitwieser, G. E., and J. M. Russell, unpublished observations). Nevertheless, the anion transporter/ pH_i regulator in barnacle does respond to cyclic AMP, which indicates an ATP sensitivity.

The free $[Mg^{2+}]_i$ in barnacle muscle is reported to be in the range of 4–6 mM (Ashley and Ellory, 1972; Brinley et al., 1977). Such values are in reasonable agreement with $[Mg^{2+}]_i$ values reported for other cells of between 0.5 and 4 mM (e.g., De Weer, 1976; Baylor et al., 1982; Alvarez-Leefmans et al., 1984). Such an $[Mg^{2+}]_i$ is sufficiently high to exert significant inhibition on the pH_i regulator/anion transporter, especially at pH_i values that are only mildly acidic (see Fig. 6). Thus, Mg^{2+} may be responsible for the “set-point” of the pH_i regulator being at a more acidic pH_i than expected from purely thermodynamic considerations (Russell et al., 1983).

Summary

We have described the effects of two cations, H^+ and Mg^{2+} , on an anion-transport mechanism responsible for the regulation of pH_i . The Cl^- transporter is exquisitely sensitive to changes of pH_i , being stimulated to transport Cl^- 10–20 times faster by a fall of pH_i from the normal value of 7.35 to a value of 6.8. This stimulatory effect of $[H^+]_i$ can be reduced by increases of $[Mg^{2+}]_i$ in such a way that it appears the two cations may compete for common sites; when they are occupied by H^+ , this competition results in activity by the transporter. Mg^{2+} appears to hinder such occupation and thereby prevent the activation of the transporter.

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