Endogenous K^+/H^+ Exchange Activity in the Sf9 Insect Cell Line†

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ABSTRACT: The fluorescent pH indicator 2′,7′-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF) was used to investigate changes in the intracellular pH (pH_i) of individual Sf9 cells in response to changes in the composition of the external medium. Under standard conditions, the resting pH_i was 0.2−0.3 unit lower than the extracellular pH (6.5). The extracellular concentration of K^+ had a major influence on pH_i. Removal of K^+ from the medium resulted in a rapid but reversible acidification of the cells. The buffer capacity of the cells was a U-shaped function of pH_i with a minimum at about $pH_i = 6.2$. In the presence of K^+, a change in the pH of the medium was followed by an equivalent change in pH_i. In its absence, however, changes in the external pH had little effect on the pH of the cells. Following removal of K^+ from the medium, the cells realkalinized at an initial rate which increased with increasing concentration of added K^+. This cation was about 30 times more effective in promoting realkalinization of the cells than Li^+, Na^+, Rb^+, and Cs^+. The apparent K_m for K^+-dependent H^+ efflux was about 12 mM and was slightly modulated by extracellular pH. These results strongly suggest that, in Sf9 cells, a K^+/H^+ antiporter plays a key role in the movement of protons across the cell membrane.

Sf9 and Sf21 cells, derived from the ovaries of the fall armyworm *Spodoptera frugiperda*, are best known as the preferred host cells for the replication of the nuclear polyhedrosis baculovirus from *Autographa californica* (Cameron et al., 1989). This and other related baculoviruses have become very useful viral vectors for the high-level expression of foreign genes in eukaryotes (Kang, 1988; Luckow & Summers, 1988; Miller, 1988; Fraser, 1992). Recently, a number of integral membrane proteins have been functionally expressed and characterized in Sf9 cells, including a *Drosophila* Shaker K^+ channel (Klaiber et al., 1990), the cystic fibrosis gene product (Kartner et al., 1991), a human K^+ channel (Kamb et al., 1992), a human Na^+/H^+ antiporter (Fafournoux et al., 1991), canine cardiac (Li et al., 1992) and human renal (Loo & Clarke, 1994) Na^+/Ca^{2+} exchangers, a rabbit intestinal Na^+/glucose cotransporter (Smith et al., 1992), a rat anion exchanger (He et al., 1993), a rat Na^+- and Cl^--dependent serotonin transporter (Tate & Blakely, 1994), a rat renal Na^+/phosphate cotransporter (Fucentese et al., 1995), the human P-glycoprotein associated with multidrug resistance (Germann et al., 1990; Sarkadi et al., 1992; Rao et al., 1994), a human plasma membrane Ca^{2+}-ATPase (Heim et al., 1992), different isoforms of the rodent Na^+/K^+-ATPase (De Tomaso et al., 1993; Blanco et al., 1993), a gastric H^+/K^+-ATPase (Klaassen et al., 1993), and a large number of cell-surface receptors.

These studies have taken advantage of the absence or low level of expression of these different activities in insect cells. However, in spite of this extensive use of Sf9 cells for the study of foreign membrane proteins, their endogenous transport proteins have received little attention, except for the presence of low-conductance chloride channels (Schwartz et al., 1991; Gabriel et al., 1992) and equilibrative nucleoside transporters (Hogue & Cass, 1994). A more detailed understanding of the physiology of Sf9 cells would undoubtedly contribute to further increase their usefulness as an heterologous expression system. In addition, because they are easy to grow and well suited for patch-clamp and microfluorescence studies (Schwartz et al., 1991; Kamb et al., 1992), Sf9 cells should constitute a favorable model for examining different aspects of insect cell physiology.

Na^+/H^+ antiporters are ubiquitous in both prokaryotes and eukaryotes where they play an essential role in a variety of cellular processes such as intracellular pH regulation, osmoregulation, and generation of a sodium-motive force across the cell membrane (Aronson, 1985; Padan & Schuldiner, 1993). Neither pH regulation nor cell volume regulation has been studied extensively in insect cells, but a K^+/H^+ exchanger (Wieczorek et al., 1991; Lepier et al., 1994) coupled with a vacuolar-type H^+-ATPase (Wieczorek et al., 1989) has been implicated in the generation of the potassium electrochemical gradient which is responsible for secondary active transport across the apical membrane of midgut enterocytes (Giordana et al., 1982; Chao et al., 1991; Sacchi et al., 1994).

In the present study, we have examined the effect of different changes in the composition of the extracellular medium on the intracellular pH (pH_i)[1] of Sf9 cells. Removal of K^+ from the medium resulted in a reversible acidification

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[1] Abbreviations: BCECF, 2′,7′-bis(carboxyethyl)-5,6-carboxyfluorescein; pH_i, intracellular pH; Pipes, 1,4-piperazinediethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid; SITS, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid.

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of the cells. Since both pH_i and the rate of proton transport were markedly influenced by the extracellular K^+ concentration, our results strongly suggest the presence of a strong and highly specific K^+/H^+ antiport activity in the plasma membrane of these insect cells.

EXPERIMENTAL PROCEDURES

Cell Cultures. Sf9 cells (ATCC CRL 1711) were grown at 27 °C in Grace's insect cell culture medium (Gibco, Gaithersburg, MD) (Grace, 1962) supplemented with 350 mg/L sodium bicarbonate, 3.33 g/L yeastolate (Difco, Detroit, MI), 3.33 g/L lactalbumin hydrolysate (Difco), 50 mg/L sodium ampicillin (Gibco), and 10% (v/v) heat-inactivated fetal bovine serum (Gibco) (Hink, 1979). Cultures (100−150 mL) were carried out in spinner flasks (Bellco Glass, Vineland, NJ) inoculated with 1.0×10^5 cells/mL with constant stirring at 50−60 rpm. Before use, cells were allowed to settle onto circular 22-mm diameter no. 1 glass coverslips (Fisher Scientific, Montreal) for at least 30 min.

Solutions. All chemicals were obtained from Sigma (St. Louis, MO), unless noted otherwise. Experiments were carried out in 50 mM KCl, 21 mM NaCl, 14 mM $MgCl_2$, 11 mM $MgSO_4$, 6.8 mM $CaCl_2$, 3.9 mM glucose, 2.2 mM fructose, 120 mM sucrose, and 10 mM 1,4-piperazinediethanesulfonic acid (Pipes)/Tris (pH 6.5). This solution was designed to approximate the cationic composition of Grace's growth medium and was therefore designated as G* medium. Its pH and composition were modified as required for individual experiments. When KCl or NaCl were omitted, or when their concentration was reduced, they were replaced by *N*-methyl-D-glucamine hydrochloride to maintain the ionic strength of the solutions constant. The osmolality of all solutions was measured with a DigiMatic Model 3D2 osmometer (Advanced Instruments, Needham Heights, MA) and adjusted to 370 mOsmol/kg of H_2O with sucrose.

Intracellular K^+ Content. The intracellular K^+ concentration was estimated by flame photometry after separation of the cells from the incubation medium by centrifugation through an oil layer. Cells [$(5-10) \times 10^6$/mL)] in Grace's medium (density = 1.002 g/mL) containing 0.5 mM [^{3}H]-mannitol (New England Nuclear, Boston, MA) (15 μCi/mL) were placed on top of an oil layer (density = 1.025 g/mL) composed of 5 parts of *n*-bromodecane and 1 part of silicone oil overlaying a sucrose solution (density = 1.038 g/mL) containing 0.1% (w/v) Triton X-100. After centrifugation at 8000*g* for 1 min at room temperature, the incubation medium and oil layer were removed carefully, and an aliquot of the sucrose solution, which included the cytosolic content of the lysed cells, was taken for the measurement of K^+. In parallel determinations, total cell volume was estimated with a Model ZBI Coulter Counter (Coulter Electronics, Hialeah, FL). The intracellular K^+ concentration was calculated by dividing total cellular K^+ by total cell volume after correction for contamination by the incubation medium, estimated from the amount of [^{3}H]mannitol in the sucrose layer and determined by liquid scintillation counting.

Intracellular pH Measurements and Calibration. Cells were loaded by incubation with 5 μM 2′,7′-bis(carboxyethyl)-5,6-carboxyfluorescein acetoxymethyl ester (BCECF/AM), the membrane-permeant derivative of BCECF (Molecular Probes, Eugene, OR), in G* medium for 20 min at room temperature (20−23 °C). During this period, the fluorescence of the cells rose gradually and approached a plateau as BCECF/AM was converted into the membrane-impermeant acid form of BCECF by the action of cytoplasmic esterases (Kaunitz, 1988). The cells were then rinsed with BCECF-free G* medium. Experiments were carried out at room temperature in a custom-made coverslip holder fitted to the stage of an Olympus IMT-2 inverted microscope (Olympus Optical Co., Tokyo) coupled to a PTI spectrofluorometer (Photon Technology International, South Brunswick, NJ). Measurements of the fluorescence of single cells were done at 450- and 500-nm excitation wavelengths alternating at a frequency of 100 Hz. The sample was illuminated through a 40×-epifluorescence objective (UV-FL40, N.A. 0.85, Olympus). The emitted light was collected by the same objective and passed through a 530-nm interference filter (10-nm bandwidth), and its intensity was recorded by a photon-counting photomultiplier detector mounted on the microscope. Background fluorescence was subtracted from the raw data, and data points were recorded every 1 s.

At the end of each experiment, the dependence of the intracellular BCECF fluorescence ratio on pH_i was calibrated with the nigericin-high K^+ technique (Thomas et al., 1979). pH_i was varied from about 5.25 to 7.75 by incubating the cells successively in a series of solutions containing 150 mM KCl, 2 mM $MgCl_2$, 6 μM nigericin, and 10 mM of either 4-morpholineethanesulfonic acid (Mes)/Tris (pH 5.25−6.0) or Pipes/Tris (pH 6.25−7.75).

Intracellular Buffer Capacity. The intracellular buffer capacity of Sf9 cells was estimated with the technique described by Boyarsky et al. (1988). After removal of K^+ from the external medium, the cells were exposed to 60 mM NH_4Cl. The external medium was then replaced by a series of solutions with decreasing NH_4Cl concentration. The intracellular buffering capacity ($\Delta[NH_4^+]_i/\Delta pH_i$) was calculated from the resulting steps in intracellular NH_4^+ concentration and pH_i caused by the rapid equilibration of NH_3 across the cell membrane (Boyarsky et al., 1988).

RESULTS

BCECF Fluorescence Ratio as a Function of Intracellular pH. Sf9 cells were grown in Grace's insect cell culture medium at pH 6.4, and the pH of the media used in the experiments described herein ranged from 6.0 to 7.0. Under these conditions, the intracellular pH often falls below the range where the fluorescence ratio of BCECF is a linear function of pH. BCECF can nevertheless be used to obtain accurate measurements of pH_i down to almost pH 5 with careful calibration (Restrepo et al., 1990). Figure 1A shows a representative calibration experiment for a BCECF-loaded Sf9 cell. The ratio of the fluorescence intensities was measured for the excitation wavelengths of 500 and 450 nm after exposure of the cells to different pH values in the presence of nigericin. The data were fitted with a parabola (Figure 1B) which was used to convert the measured fluorescence ratios into pH_i values:

$$F_{500}/F_{450} = a[(\mathrm{pH})^2 + b(\mathrm{pH}) + c]$$

Although a different calibration curve was established for each experiment to take small cell-to-cell variations into account, the parameters obtained for different experiments

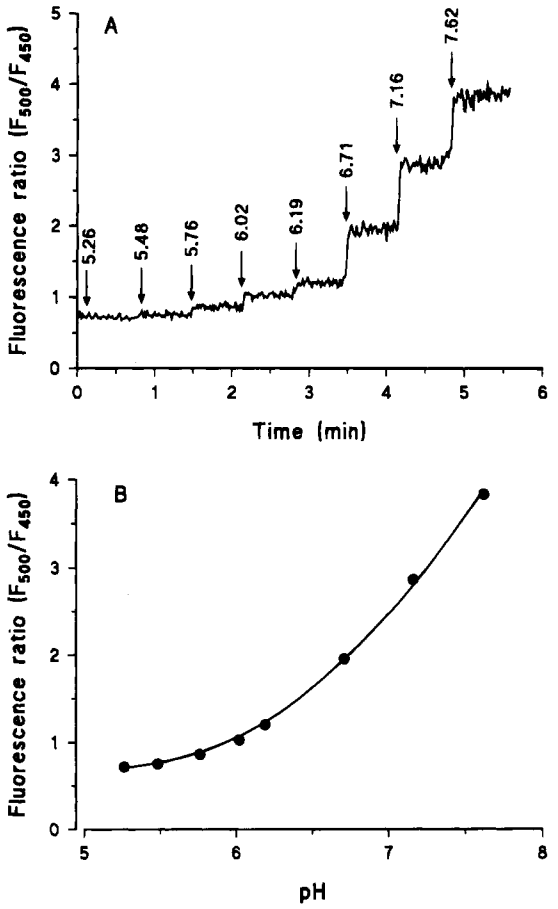

FIGURE 1: Dependence of BCECF fluorescence ratio on pH_i. At the end of each experiment, the cells were incubated in solutions containing 6 μM nigericin at the indicated pH values, and the fluorescence ratio was recorded for the excitation wavelengths of 500 and 450 nm at the emission wavelength of 530 nm. (A) Typical tracing. (B) Calibration curve obtained by plotting the average fluorescence ratio at each pH value. The line was obtained by fitting a parabola to the points.

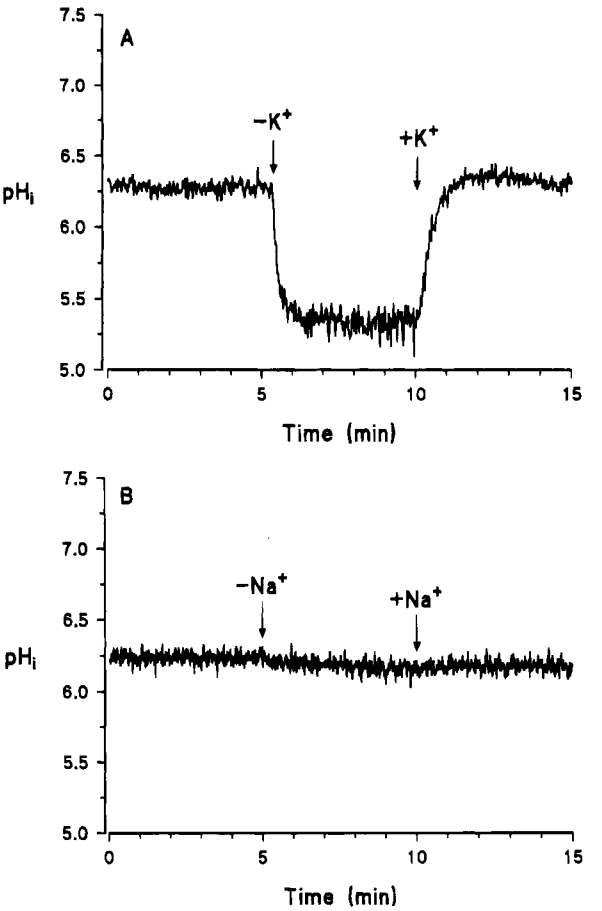

FIGURE 2: Effect of removing K^+ and Na^+ from the external medium on the intracellular pH of Sf9 cells. Cells were incubated in G* medium (see Experimental Procedures). During the indicated intervals, KCl (A) and NaCl (B) were replaced by *N*-methyl-D-glucamine hydrochloride to maintain the ionic strength and osmolarity of the medium constant.

were remarkably constant: $a = 0.623 \pm 0.021$, $b = -10.273 \pm 0.069$, and $c = 27.77 \pm 0.34$ (means $\pm$ SEM for 38 experiments).

The intracellular K^+ concentration was estimated at 129 $\pm$ 12 mM (mean $\pm$ SEM for 8 experiments) by flame photometry. This value is based on total cellular K^+ content and total cell volume. It must therefore be considered as an approximation of the actual cytosolic K^+ concentration. Because nigericin mediates K^+/H^+ exchange across the plasma membrane, pH_i may differ significantly from the pH of the calibration solution if the intracellular and extracellular K^+ concentrations differ widely during calibration (Thomas et al., 1979; Chaillet & Boron, 1985; Negulescu & Machen, 1990). To test this possibility, 5 μM valinomycin was added to the calibration solutions to increase the permeability of the cell membrane for K^+ ions (results not shown). Valinomycin had little effect on the fluorescence ratios measured at different pH values, however, suggesting that our calibration curves allow a reliable estimation of pH_i.

Effect of Extracellular K^+ Concentration on Intracellular pH. The intracellular pH of Sf9 cells depended strongly on the presence or absence of extracellular K^+, the most abundant monovalent cation in Grace's medium (Grace, 1962; Hink, 1979) and the hemolymph of lepidopteran insects (Florkin & Jeuniaux, 1974). The resting pH_i of Sf9 cells in G* medium (pH 6.5) was 6.28 $\pm$ 0.01 (mean $\pm$ SEM of 30 experiments). Replacing KCl by *N*-methyl-D-glucamine hydrochloride in the incubation medium resulted in a rapid acidification of the cells to pH 5.41 $\pm$ 0.01 ($n = 30$) (Figure 2A). pH_i returned rapidly to its original level when the cells were returned to G* medium. In contrast, removal of NaCl from the extracellular medium had little effect on pH_i (Figure 2B).

Changes in the extracellular concentration of K^+ induced rapid changes in pH_i (Figure 3A). The difference between final and initial pH_i levels (ΔpH_i) was linearly related to the logarithm of the ratio of final to initial extracellular K^+ concentrations with a slope of 0.677 (Figure 3B). The initial rate of alkalinization following an increase in extracellular $[K^+]$ was significantly lower than the initial rate of acidification following an equivalent decrease in extracellular K^+ concentration (Figure 3C). The rates of change in pH_i (dpH_i/dt) reported here can be used directly as estimates of proton fluxes across the cell membrane, in spite of a variation in buffer capacity (see below), since, in a given experiment, all measurements were done at the same initial pH_i.

Intracellular Buffer Capacity. The buffer capacity of Sf9 cells varied markedly with pH_i within the range of pH 5.5–6.5 (Figure 4). A minimum value was observed near pH 6.2 which, as mentionned above, corresponds approximately to the resting pH_i of Sf9 cells in G* medium.

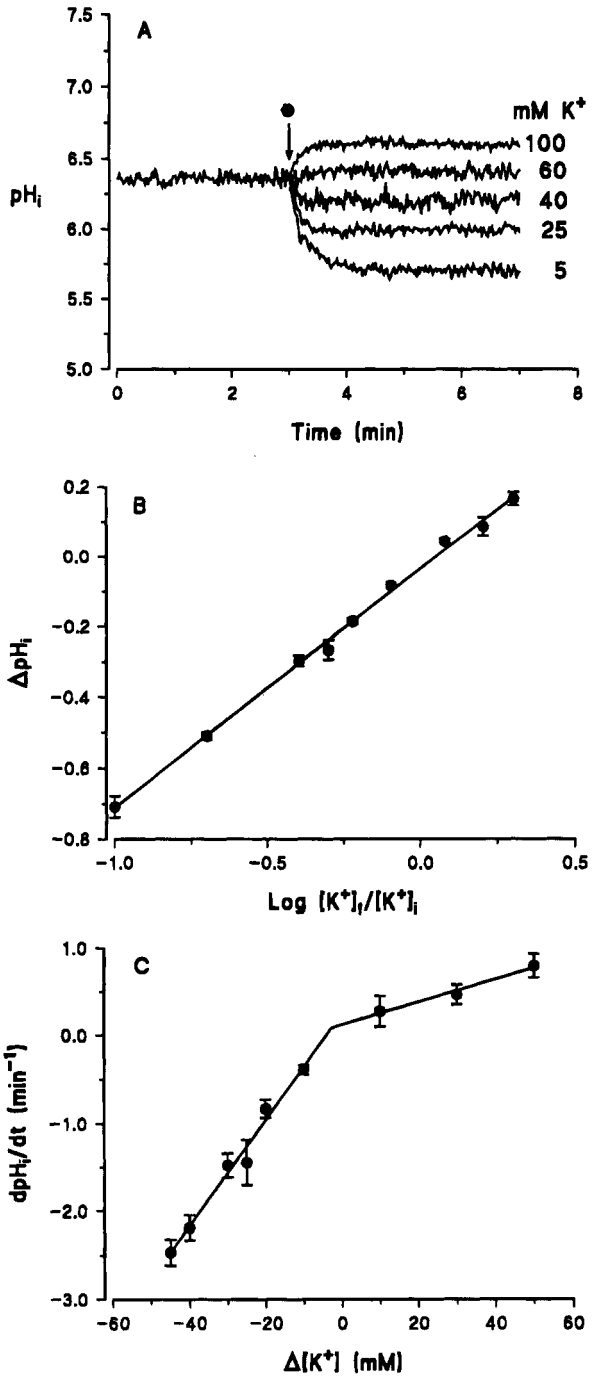

FIGURE 3: Effect of changes in the extracellular concentration of K^+ on the intracellular pH of Sf9 cells. Cells were incubated in G* medium (pH 6.5) containing 50 mM KCl. At the indicated time (*), the medium was replaced by solutions containing different concentrations of K^+. (A) Typical tracings. (B) Compilation of the changes in pH_i at different K^+ concentrations (r = 0.998). (C) Compilation of the initial rates of change in the intracellular pH following changes in the K^+ concentration of the medium. Values are means ± SEM for 3−12 independent experiments.

Effect of Extracellular pH on Intracellular pH. In the presence of 50 mM K^+, changes in the pH of the medium were followed by equivalent changes in pH_i. Within about 1 min, pH_i reached a steady level at 0.2−0.3 pH unit below the pH of the bathing solution at pH 6.0, 6.5, and 7.0 (Figure 5). In contrast, after the initial acidification following removal of K^+ from the medium, similar changes in the extracellular pH had little effect on the pH of the cells,

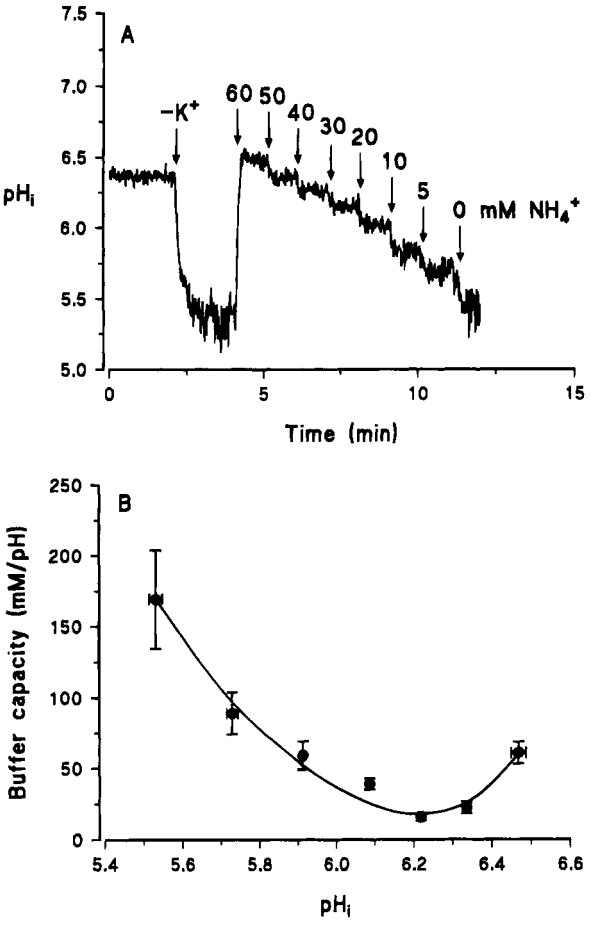

FIGURE 4: pH dependence of the buffer capacity of Sf9 cells. After removal of K^+ from the external medium (pH 6.5) to inhibit the K^+/H^+ exchange activity, pH_i was varied by exposing the cells to different NH_4Cl concentrations. The buffer capacity was calculated from the resulting shifts in intracellular pH precisely as described in detail by Boyarsky et al. (1988). (A) Typical tracing. (B) Compilation of the buffer capacity measured at different pH_i values. The indicated pH_i values correspond to the midpoint between the average pH_i measured before and after each step. Values are means ± SEM for 5 independent experiments.

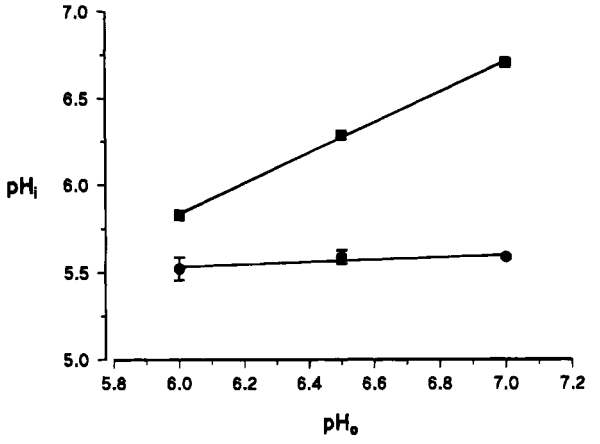

FIGURE 5: Effect of variations in the external pH (pH_o) on the intracellular pH of Sf9 cells in the presence of extracellular K^+ (■) and following the removal of K^+ from the medium (●). Experiments were carried out in G* medium at the indicated pH values. When omitted, KCl was replaced by *N*-methyl-D-glucamine hydrochloride. Values are means ± SEM for 3 independent experiments.

although pH_i was consistently observed to rise slowly after several minutes of incubation in the absence of extracellular

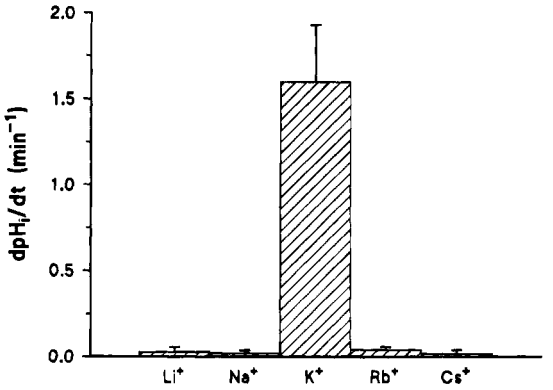

FIGURE 6: Ionic selectivity of K^+/H^+ exchange in Sf9 cells. Cells were acidified by replacing KCl by *N*-methyl-D-glucamine hydrochloride in the bathing medium. The latter was then replaced by the chloride salts of either Li^+, Na^+, K^+, Rb^+, or Cs^+. Values are means ± SEM of the initial rates of realkalinization for 4 independent experiments.

K^+ (Figure 5). This slight alkalinization was independent of the extracellular pH and was observed in the absence of changes in external pH or when such changes were done in the reverse order.

Ionic Selectivity. Because extracellular K^+ had a major influence on pH_i, its ability to promote realkalinization of the cells following their acidification, caused by incubation in K^+-free medium, was compared with that of other monovalent cations (Figure 6). Under these conditions, the initial rate of recovery induced by K^+ was about 30-fold higher than those observed after addition of Na^+ or other alkali metal ions.

Effect of Extracellular K^+ Concentration and pH on the Rate of pH_i Recovery. The kinetics of pH_i recovery after acidification of the cells by removal of K^+ from the bathing medium were examined at external pH = 6.0, 6.5, and 7.0 (Figure 7). Because pH_i was independent of extracellular pH in the absence of extracellular K^+, the initial pH gradient across the cell membrane differed greatly for different extracellular pH values. The rates of H^+ efflux were therefore normalized using a protocol similar to that proposed by Casavola et al. (1991) to reduce the cell-to-cell variability in absolute transport rates measured in single cells. As illustrated in Figure 7A, after the acidification of the cells, a small amount of K^+ was added to raise the K^+ concentration of the bath moderately followed by elevation of $[K^+]$ to 50 mM. For each pH value, the rates of realkalinization at different K^+ concentrations were then normalized to the rate measured in the presence of 50 mM K^+. Care was taken to replace the first K^+-containing solution, in different experiments, once a similar pH_i (5.5−5.6) had been reached.

The rate at which the cells alkalinized in the presence of K^+ depended on both the K^+ concentration and pH of the external medium (Figure 7B). At pH 7.0, the rate of H^+ efflux followed simple Michaelis−Menten kinetics, and the data could be fitted with a straight line in a Lineweaver−Burk plot (not shown). At pH 6.5 and 6.0, however, the K^+ dependence of H^+ efflux was sigmoidal. The K^+ concentrations at which the half-maximal rate of alkalinization occurred were about 7, 12, and 17 mM at pH 7.0, 6.5, and 6.0, respectively. The Hill coefficient for proton efflux was 2.2 at pH 7.0, 2.4 at pH 6.5, and 2.5 at pH 6.0.

Effect of Transport Inhibitors. The following substances did not affect significantly the intracellular pH of Sf9 cells,

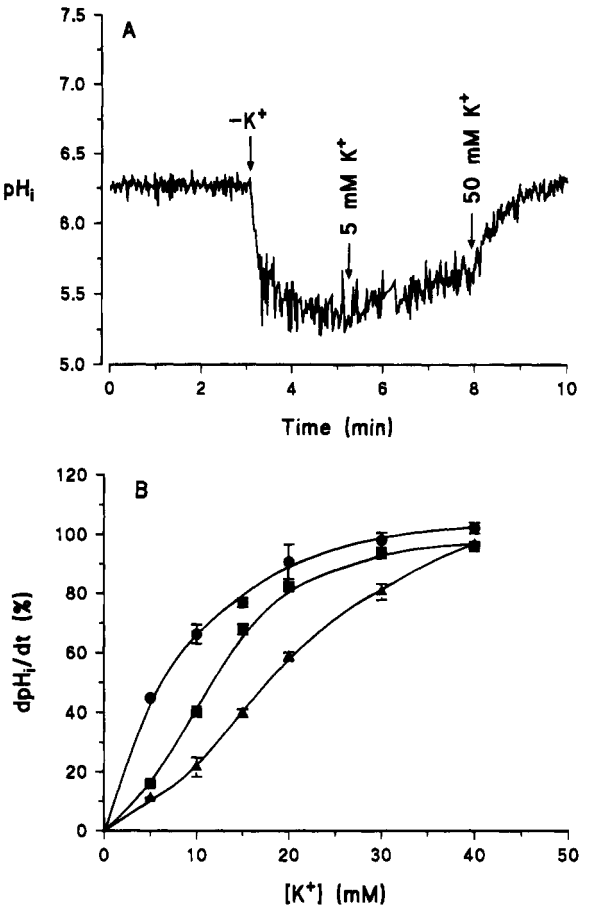

FIGURE 7: Effect of K^+ concentration and extracellular pH on the rate of H^+ efflux from Sf9 cells. Cells were acidified by removing extracellular K^+ and realkalinized by adding different K^+ concentrations to the bathing medium. For each experiment, a given concentration of K^+ (5−40 mM) was added first, followed by 50 mM K^+ at the same pH. The initial rates of H^+ efflux were normalized to the values obtained after addition of 50 mM K^+. (A) Typical tracing obtained at extracellular pH = 6.5. (B) Normalized rates of H^+ efflux as a function of the K^+ concentration at extracellular pH = 6.0 (▲), 6.5 (■), and 7.0 (●).

nor their rate of acidification in response to removal of K^+ from the external medium, or the rate of pH recovery following their return to a 50 mM K^+-containing medium: $BaCl_2$ (5 mM), $ZnCl_2$ (5 mM), lidocaine (5 mM), oligomycin (5 mM), sodium azide (2 mM), sodium vanadate (1 mM), SCH28280 (50 μM), strophantidin (1 mM), bafilomycin (0.1 μM), amiloride (1 mM), harmaline (100 μM), and 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS) (1 mM). For experiments with $BaCl_2$ and $ZnCl_2$, $MgSO_4$ was omitted from the media (replaced by sucrose) to avoid the formation of precipitates. This change in medium composition did not affect pH_i of Sf9 cells in the presence or absence of K^+ (not shown). In contrast, quinine, a known inhibitor of the mitochondrial K^+/H^+ antiporter (Nakashima & Garlid, 1982), decreased the initial rate and extent of cellular acidification in a concentration-dependent manner (Figure 8A). However, in the presence of quinine and following removal of K^+ from the external medium, changes in the extracellular pH resulted in sharp shifts in pH_i (Figure 8B), suggesting that, in Sf9 cells, quinine may act by increasing the permeability of the cell membrane to H^+ ions rather than by directly inhibiting a K^+/H^+ exchange activity. Nevertheless, this increased permeability did not appear to cause the collapse of all ionic gradients and electrical potential across

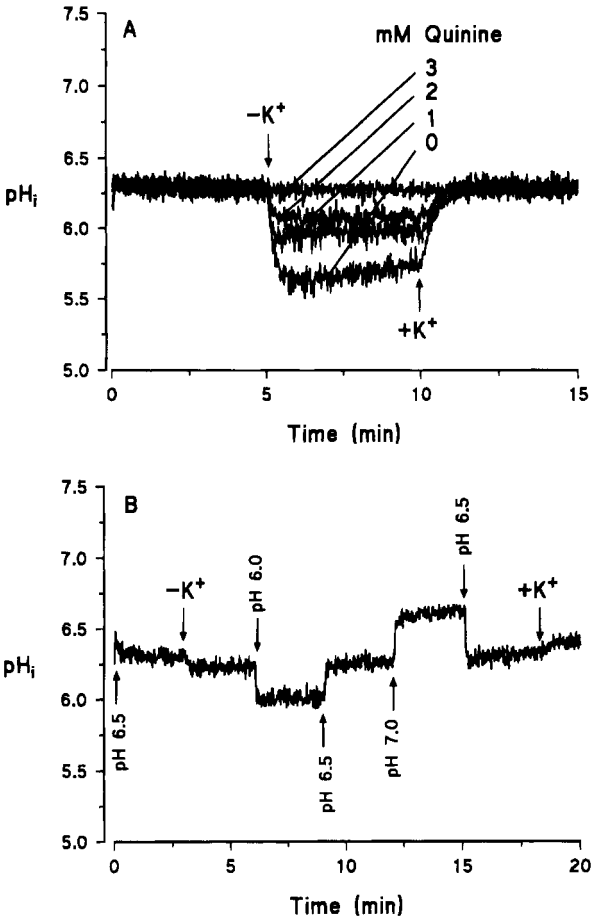

FIGURE 8: Effect of quinine on the intracellular pH of Sf9 cells. (A) Cells were incubated in G* medium, pH 6.5. During the indicated interval, KCl was replaced by *N*-methyl-D-glucamine hydrochloride. Quinine was maintained at the indicated concentrations throughout each experiment. (B) Cells were incubated with 3.0 mM quinine, and the extracellular pH was varied as indicated after removal of K^+ from the external medium. Representative tracings are shown.

the plasma membrane since, except at pH = 6.0, pH_i stabilized well below the extracellular pH (Figure 8B).

DISCUSSION

The results of the present study clearly demonstrate that the cytoplasmic pH of Sf9 cells depends strongly on the extracellular concentration of K^+ ions. The rapid and reversible acidification of the cells which follows removal of K^+ from the external medium could, at least in principle, be attributed to the presence of either (1) two parallel conductive pathways for K^+ and H^+ ions, respectively; (2) a K^+/H^+-ATPase or a Na^+/K^+-ATPase coupled with a Na^+/H^+ exchanger; or (3) a K^+/H^+ exchanger in the cell membrane. Among these possibilities, however, the presence of a K^+/H^+ exchanger apears to be, by far, the most likely in light of the available evidence.

In the first hypothesis, efflux of K^+ in the absence of an accompanying anion would cause an influx of H^+ to compensate for the resulting intracellular deficit in positively charged ions. Changes in pH_i were not affected, however, by the K^+ channel inhibitors Ba^{2+}, Zn^{2+}, and lidocaine. Sf9 cells are also known to have, at most, very few K^+ channels (Klaiber et al., 1990; Schwartz et al., 1991; Kamb et al., 1992). This scarcity of K^+ channels cannot be attributed to some unknown factor which would prevent channel activity from being detected with the patch-clamp technique since endogenous chloride channels (Schwartz et al., 1991; Gabriel et al., 1992) and foreign K^+ channels (Klaiber et al., 1990; Kamb et al., 1992) expressed in Sf9 cells are readily detectable. In addition, K^+ channels are easily detected in the closely related UCR-SE-1a cell line derived from *Spodoptera exigua* (Monette et al., 1994).

According to the second hypothesis, the cellular acidification induced by removal of K^+ ions from the external medium would result from the inhibition of a proton extrusion system involving a K^+-dependent ATPase. This possibility also appears unlikely since changes in pH_i were not affected by the addition of inhibitors of ATP biosynthesis including oligomycin and sodium azide or inhibitors of ATP hydrolysis including sodium vanadate, SCH28280 (inhibits K^+/H^+-ATPases), strophantidin (inhibits Na^+/K^+-ATPases), and bafilomycin (inhibits vacuolar-type H^+-ATPases). Sf9 cells have also been shown to have undetectable levels of K^+/H^+-ATPase (Klaassen et al., 1993) and very low levels of Na^+/K^+-ATPase (De Tomaso et al., 1993; Blanco et al., 1993) activity. In addition, the intracellular pH was essentially unaffected by changes in the extracellular concentration of Na^+, thus excluding the participation of a Na^+/H^+ exchanger. The absence of Na^+/H^+ exchange activity in Sf9 cells has also been reported previously (Fafournoux et al., 1991).

Finally, the third hypothesis, besides being consistent with the results of the present study, is supported by the presence of a K^+/H^+ exchanger in at least another insect cell type (Wieczorek et al., 1991). Both exchangers differ, however, in a number of respects. While the K^+/H^+ exchanger from midgut goblet cells of *Manduca sexta* was efficiently inhibited by amiloride and harmaline (Wieczorek et al., 1991), that of Sf9 cells was unaffected. K^+/H^+ exchangers appear to differ widely in their susceptibility to various inhibitors. For example, those of the rat ileum brush-border membrane (Binder & Murer, 1986) and rabbit gastric gland (Hofer & Machen, 1992) are not affected by amiloride, in contrast with the Na^+/H^+ exchangers of these tissues. Also, the gastric gland K^+/H^+ exchanger was inhibited by high concentrations of SCH28280 (Hofer & Machen, 1992), but this compound had no effect on the K^+/H^+ exchange activity of Sf9 cells.

The different susceptibilities of both insect K^+/H^+ exchangers to amiloride and harmaline, an inhibitor of Na^+-dependent symporters, could possibly be related to their difference in ionic selectivity. While, in the case of the midgut exchanger, Rb^+, Li^+, and Na^+ could substitute for K^+ to varying degrees, the K^+/H^+ exchange activity of Sf9 cells was highly specific for K^+ ions. In fact, this high degree of specificity for K^+ appears to be rather unique when compared with those of bacterial (Brey et al., 1980) and animal (Binder & Murer, 1986; Cala et al., 1986; Hofer & Machen, 1992) plasma membrane K^+/H^+ exchangers for which other alkali metal ions can also function as substrates, albeit with different efficiencies.

Although extracellular pH was found to modulate the activity of the K^+/H^+ exchanger of Sf9 cells, the influence of intracellular pH remains unknown. Unfortunately, the study of this parameter was precluded by the very strong dependence of pH_i on the extracellular K^+ concentration. Attempts to cause variations in pH_i, without varying the

extracellular K^+ concentration, using an NH_4Cl prepulse technique (Boron & DeWeer, 1976) were unsuccessful. In the absence of K^+, or when K^+ and NH_4Cl were removed simultaneously from the external medium, the pH_i of cells incubated with NH_4Cl dropped to a constant level upon removal of the NH_4Cl independently of the ammonium concentration used. In the presence of K^+, the cells realkalinized very rapidly following removal of NH_4Cl so that the initial pH_i and rate of H^+ efflux could not be determined accurately.

Because pH_i was strongly influenced by abrupt changes in the extracellular K^+ concentration, an effect of the electrical membrane potential on the K^+/H^+ exchange activity cannot be excluded. A strong effect of the membrane potential could also contribute to the exchanger's specificity for K^+ ions. The *M. sexta* midgut K^+/H^+ exchanger is thought to be electrogenic with more than one H^+ being transported for each K^+ (Wieczorek et al., 1991; Lepier et al., 1994). In fact, this property appears to be necessary for explaining its involvement in active K^+ secretion (Chao et al., 1991; Wieczorek et al., 1991) although this conclusion has been questioned (Dow, 1992). The possibility that the K^+/H^+ exchanger could also be electrogenic in Sf9 cells may be suggested by the fact that the Hill coefficients (Segal, 1975) estimated from the rates of K^+-dependent H^+ efflux were larger than 1. Further work including measurements of the membrane potential and rates of K^+ transport will be necessary to clarify this point and establish a precise stoichiometry for this exchange activity.

The physiological role of a plasma membrane K^+/H^+ exchanger in Sf9 cells remains to be ascertained. Because these cells are derived from ovarian tissue, they may not be expected to have strong mechanisms for adapting to variations in extracellular pH or K^+ concentration. In fact, even in the presence of K^+, Sf9 cells were clearly unable to maintain a constant intracellular pH in response to changes in extracellular pH. They did, however, maintain a constant pH gradient of 0.2−0.3 pH unit across their cell membranes. At present, it is unclear whether the K^+/H^+ exchanger of Sf9 cells is involved in the generation of a K^+-motive force similar to that which energizes secondary active transport of substrates in the insect midgut (Wieczorek et al., 1989, 1991; Chao et al., 1991). In Sf9 cells, the intracellular K^+ concentration was much higher than that of the medium so that the K^+ gradient appears to be oriented in the opposite direction. Further work on the membrane transport mechanisms and bioenergetics of Sf9 cells is therefore required to establish the role of their K^+/H^+ exchanger. Such studies appear especially worthwhile in view of the fact that membrane transport phenomena in insect cells have so far been almost exclusively investigated in epithelial and neuronal tissues.

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