# Na$^+$, K$^+$, and Cl$^-$ Transport in Resting Pancreatic Acinar Cells

HONG ZHAO and SHMUEL MUALLEM

From the Department of Physiology, University of Texas Southwestern Medical Center, Dallas, Texas 75235

ABSTRACT   To understand the role of Na$^+$, K$^+$, and Cl$^-$ transporters in fluid and electrolyte secretion by pancreatic acinar cells, we studied the relationship between them in resting and stimulated cells. Measurements of $[Cl^-]_i$ in resting cells showed that in HCO$_3^-$-buffered medium $[Cl^-]_i$ and Cl$^-$ fluxes are dominated by the Cl$^-$/HCO$_3^-$ exchanger. In the absence of HCO$_3^-$, $[Cl^-]_i$ is regulated by NaCl and NaK2Cl cotransport systems. Measurements of $[Na^+]_i$ showed that the Na$^+$-coupled Cl$^-$ transporters contributed to the regulation of $[Na^+]_i$, but the major Na$^+$ influx pathway in resting pancreatic acinar cells is the Na$^+$/H$^+$ exchanger. $^{86}$Rb influx measurements revealed that >95% of K$^+$ influx is mediated by the Na$^+$ pump and the NaK2Cl cotransporter. In resting cells, the two transporters appear to be coupled through $[K^+]_i$ in that inhibition of either transporter had small effect on $^{86}$Rb uptake, but inhibition of both transporters largely prevented $^{86}$Rb uptake. Another form of coupling occurs between the Na$^+$ influx transporters and the Na$^+$ pump. Thus, inhibition of NaK2Cl cotransport increased Na$^+$ influx by the Na$^+$/H$^+$ exchanger to fuel the Na$^+$ pump. Similarly, inhibition of Na$^+$/H$^+$ exchange increased the activity of the NaK2Cl cotransporter. The combined measurements of $[Na^+]_i$ and $^{86}$Rb influx indicate that the Na$^+$/H$^+$ exchanger contributes twice more than the NaK2Cl cotransporter and three times more than the NaCl cotransporter and a tetraethylammonium-sensitive channel to Na$^+$ influx in resting cells. These findings were used to develop a model for the relationship between the transporters in resting pancreatic acinar cells.

## INTRODUCTION

Pancreatic acinar cells secret digestive enzymes and isotonic fluid rich in NaCl (Schulz, 1987; Case, 1989). Whereas enzyme secretion (Gardner and Jensen, 1986; Williams and Blevins, 1993) stimulus-secretion coupling (Hootman and Williams, 1987; Petersen and Findlay, 1987) and second messenger regulation (Muallem, 1992) have been studied extensively, the mechanism of fluid and electrolyte secretion by acinar cells is poorly understood.

Address correspondence to Shmuel Muallem, Ph.D., Department of Physiology, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75235.

Early studies examined the properties of fluid secretion by the perfused pancreas. In the presence of $HCO_3^-$ and cholecystokinin (CCK), where most secretion is believed to be of acinar origin, pancreatic fluid secretion was inhibited $\sim70\%$ by a high concentration of amiloride, reduction of serosal $[Na^+]$, or high concentration of acetamide isothiocyanatostilbene disulfonic acid (SITS) (Seow, Lingard, and Young, 1986; Ishikawa and Kanno, 1988, 1989). Tetraethylammonium (TEA) and $Ba^{2+}$ reduced fluid secretion by $\sim30\%$ (Evans, Pirani, Cook, and Young, 1986). Spectrofluorometric studies on the cellular level demonstrated the presence of $Na^+/H^+$ (Dufresne, Bastie, Vaysse, Creach, Hollande, and Ribet, 1985; Muallem and Loessberg, 1990a) and $Cl^-/HCO_3^-$ (Muallem and Loessberg, 1990a) exchange mechanisms in acinar cells. Strong evidence in several species points to a $Ca^{2+}$-activated $Cl^-$ channel as mediating the $Cl^-$ exit at the apical membrane (AM) (Petersen and Findlay, 1987; Peterson, 1992). In the rat and mouse pancreatic acini, in addition to $K^+$ selective channels, the basolateral membrane (BLM) contains a $Ca^{2+}$-activated, voltage-independent, nonselective cation channel that depolarizes stimulated cells (Maruyama and Petersen, 1982; Thorn and Petersen, 1992). TEA and $Ba^{2+}$ partially inhibit fluid secretion, probably by inhibition of the nonselective channels.

The ion transport pathways mentioned above, together with a paracellular transport pathway for $Na^+$, were proposed to mediate fluid and electrolyte secretion by acinar cells (Schulz, 1987; Case, 1989; Petersen, 1992). However, unlike pancreatic duct cells, acinar cells secrete fluid and electrolyte very well in the absence of $HCO_3^-$ (Kanno and Yamamoto, 1977; Petersen and Ueda, 1977; Seow et al., 1986), where their $Cl^-/HCO_3^-$ exchanger is not active (Muallem and Loessberg, 1990a,b). It was suggested that in the absence of $HCO_3^-$, $Cl^-$ entry at the BLM is mediated by the NaK2Cl cotransporter, since furosemide at high concentrations inhibited CCK-stimulated fluid secretion (Seow et al., 1986; Ishikawa and Kanno, 1989). However, a more recent report by one of these groups found no effect of serosal furosemide on fluid or $K^+$ secretion to both the luminar and the serosal fluids (Ishikawa and Kanno, 1991). Further complications arise from findings with isolated mouse pancreatic acini in which cholinergic stimulation caused ^{86}Rb and net $K^+$ efflux by the cotransporter (Singh, 1984; Petersen and Singh, 1985). Hence the existence and possible role of the NaK2Cl cotransproter in acinar cells are not clear.

In the first stage of the present studies, we attempted to determine the relationship between the $Na^+$, $K^+$, and $Cl^-$ transporters by measuring the intracellular concentrations of $Cl^-$ and $Na^+$ and the undirectional influx of [86]Rb. The results identified the major transporters regulating $[Na^+]_i$, $[K^+]_i$, and $[Cl^-]_i$ in resting acinar cells and point to a coupling between the transporters through the intracellular concentrations of $Na^+$ and $K^+$.

MATERIALS AND METHODS

*Chemicals*

Chemicals were purchased from the following sources: tributyltin, gramicidin, monensin, ouabain, (TEA) and digitonin came from Sigma Chemical Co. (St. Louis, MO). Nigericin was from Boehringer Mannheim (Indianapolis, IN). Dimethylamiloride was from Research Biochemicals Inc. (Natic, MA). Fura 2/AM, SPQ, SBFI/AM, and $Na^+$-Green/AM were from Molecular Probes

(Eugene, OR). Bumetanide was a generous gift from Dr. J. Johnson (VA Medical Center, Dallas, Texas).

### Solutions

The solutions used in the present experiments and their composition are listed in Table 1. In the text, when referring to a particular solution, we also indicate the major salt and the pH buffer as follows: solution A (NaCl, HEPES); solution B ($Cl^-$-free, HEPES); solution C ($Na^+$-free, HEPES); solution D ($K^+$-free, HEPES); solution E (NaCl, $HCO_3^-$); solution F ($Cl^-$-free, $HCO_3^-$); solution G (SCN$^-$, HEPES). All solutions containing $HCO_3^-$ were equilibrated with 5% $CO_2$/95% $O_2$ to pH 7.4 at room temperature. The osmolarity of all solutions was adjusted to 310 mosM with the major salt.

### Preparation of Pancreatic Acini

Acini were prepared according to a previously published method (Zhang, Tortorici, and Muallem, 1994). The pancreas of a 100–150-g rat was removed and injected with 10 ml of a solu-

**T A B L E   I**
*Composition of Solutions*

| Salt (mM) | Solutions | | | | | | |
|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G |
| NaCl | 145 | — | — | 145 | 120 | — | — |
| Na-Glu | — | 145 | — | — | — | 120 | — |
| NMG-CL | — | — | 145 | 5 | — | — | — |
| KCl | 5 | — | 5 | — | 5 | — | — |
| K-Glu | — | 5 | — | — | — | 5 | — |
| $MgCl_2$ | 1 | — | 1 | 1 | 1 | — | — |
| $MgSO_4$ | — | 1 | — | — | — | 1 | 1 |
| $CaCl_2$ | 1 | — | 1 | 1 | 1 | — | — |
| Ca-Cyclamate | — | 4.6 | — | — | — | 3.8 | 1 |
| HEPES | 10 | 10 | 10 | 10 | 2.5 | 2.5 | 10 |
| $NaHCO_3^-$ | — | — | — | — | 25 | 25 | — |
| Glucose | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| NaSCN | — | — | — | — | — | — | 150 |

tion composed of solution A, 0.02% soybean trypsin inhibitor, and 0.1% bovine serum albumin (PSA). Fat, connective tissue, and blood vessels were removed, the fluid was drained by blotting, and the pancreas was finely minced. The minced pancreas was added to 15 ml PSA containing 2.5 mg collagenase P and incubated for 5–6 min at 37°C. The resultant acini were washed twice with PSA and filtered through a 150-μm nylon mesh. For fluorescence measurements, acini from one pancreas were suspended in 8 ml PSA and kept on ice until use. For measurement of ^{86}Rb fluxes, the acini were suspended in 10 ml PSA and used immediately.

### Dyes Loading

For $[Ca^{2+}]_i$ measurements, 2 ml of acinar cell suspension was incubated with 2.5 μM Fura 2/AM for 20 min at 37°C. For $[Na^+]_i$ measurements, 1.5 ml acinar cell suspension was incubated with 5 μM SBFI/AM at room temperature for 45–60 min. Alternatively, 1 ml acini suspension was incubated with 2 μM $Na^+$-Green for 15 min at room temperature. At the end of each loading period, the cells were washed once with 35 ml PSA, resuspended in ~5 ml PSA, and kept on ice until plat-

ing. For each experiment, ~0.5 ml dye-loaded acini were plated on thin-glass coverslips that formed the bottom of a perfusion chamber.

Several preliminary experiments were performed to obtain suitable conditions for measurement of $[Cl^-]_i$. Acinar cells were loaded with SPQ or MQAE. In six-cell preparations, dye leak with SPQ was lower than with MQAE. Dye loading at 37°C resulted in reduced signal/noise. Incubation of the cells with SPQ at room temperature for 15 min and then at 0°C for 30 min or longer resulted in maximal loading and reproducible signal intensity between experiments performed with the same cell preparartion. Therefore, the following conditions were used to measure $[Cl^-]_i$ in acinar cells. About 3 ml acinar cell suspension was centrifuged, and the acini were resuspended in 1 ml PSA containing 10 mM SPQ. After 15 min incubation at room temperature, the acini were diluted with 1 ml PSA and kept on ice for at least 30 min and then until plating on coverslips. Before each plating, 0.1 ml of SPQ-loaded acini were added to 0.4 ml solution A in the perfusion chamber.

### Fluorescence Measurements

Dye-loaded acini were allowed to attach to the glass coverslips for ~2 min at room temperature, after which perfusion of a warm (37°C) solution A commenced to remove unattached cells. Fluorescence recording started after at least a 10-min perfusion (at a rate of 10–12 ml/min) with solution A to allow establishment of ionic gradients. The perfusion chamber was placed on the stage of an inverted microscope attached to a Delta Scan Fluorimeter, which provided a dual wavelength excitation light. Fluorescence was recorded from 2–3 cells within a single acinus composed of 10–20 cells. Fura 2 fluorescence was recorded at excitation wavelengths of 355 and 380 nm and SBFI fluorescence at 340 and 385 nm. The fluorescence of SPQ and $Na^+$-Green was recorded with single excitation wavelengths of 380 and 490 nm, respectively, since these dyes are not ratio dyes. The emitted light was directed to a photomultiplier tube by an appropriate dichroic mirror, and emission cut off filter sets. Light intensity was measured at a resolution of 2/s. The fluorescence ratios of 355/380 for Fura 2 were calibrated exactly as detailed before (Zhao, Star, and Muallem, 1994). The procedure used to calibrate the fluorescence of the other dyes is described in Results. All experimental protocols were repeated at least three times with different cell preparations.

### Measurement of $^{86}Rb$ Influx

Acini in PSA were incubated for 20 min at 37°C. To initiate $^{86}Rb$ uptake, the acini were diluted 1:1 into a warm PSA containing $^{86}Rb$ (~$4.10^5$ cpm/ml) and twice the final desired concentration of any agent tested. At the indicated times (usually after 4 min incubation at 37°C), duplicate 0.5 ml samples of acini were transferred to 8 ml stop solution containing 150 mM NaCl, 1 mM $LaCl_3$, 10 mM HEPES (pH 7.4 with NaOH), and 1 mg/ml BSA. The stop solution was in glass tubes placed in an ice-cold water bath. At the end of the experimental sampling, the acini were collected by a 20-s centrifugation at 200 g and washed twice more with an ice-cold stop solution in the same manner. The pellets were dissolved in 0.5 ml of 0.2 M NaOH by 30 min incubation at 60°C. 10–20 μl samples from representative tubes (at least six) were removed for determination of protein; the remaining volume was transferred to scintillation vials for counting. The results were calculated in terms of nmoles $K^+$/mg protein and are presented as mean ± SEM for the number of experiments performed.

### RESULTS

The first goal of the present study was to characterize the major $Cl^-$, $Na^+$, and $K^+$ transporters in acinar cells, their relative contribution to maintaining the cytosolic activities of the relevant ions, and the relationship between them. This was achieved

by measuring the intracellular activities of $Na^+$ and $Cl^-$ and the properties of $^{86}Rb$ influx.

## $Cl^-$ Transport

The intracellular $Cl^-$ concentration ($[Cl^-]_i$) was measured with the $Cl^-$-sensitive dye SPQ. Fig. 1 *a* shows the behavior of SPQ in acinar cells and the procedure used to calibrate the fluorescence. The fluorescence was relatively stable during continuous perfusion and dye leak was $\sim$12% per hour. Maximal anion-dependent fluores-
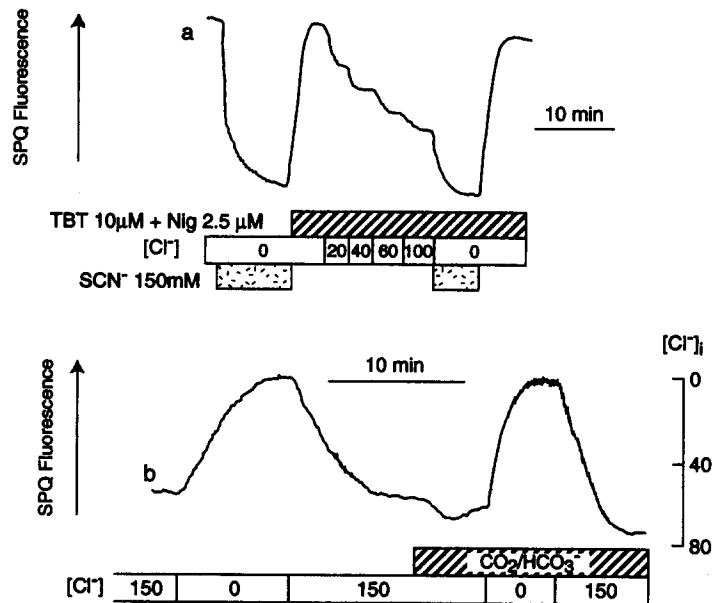


Figure 1.  Measurement of $[Cl^-]_i$ in acinar cells. Acinar cells loaded with SPQ were perfused with $Cl^-$-free solution B for 20 min before perfusion with solution G ($SCN^-$) (trace *a*). After completion of fluorescence quench, the cells were perfused with solution B containing 10 $\mu$M tributyltin and 2.5 $\mu$M nigericin and then a mixture of solution A (NaCl) and solution B to yield the indicated $[Cl^-]$. For the experiment in trace *b*, the cells were perfused with solution A, solution B, solution E ($HCO_3^-$, NaCl), or solution F ($HCO_3^-$, $Cl^-$ free). At the end of this experiment the cells were perfused with solutions F and G containing tributyltin and nigericin to determine the anion-dependent fluorescence and calculate $[Cl^-]_i$.

cence signal was obtained by exposing cells incubated in $Cl^-$-free medium to the highly permeable anion $SCN^-$. To calibrate the dye in the cells, $[Cl^-]_i$ was clamped by bathing the cells in medium containing 145 mM $K^+$ ($K_i^+ \approx K_o^+$), the $K^+/H^+$ exchange ionophore nigericin, the $Cl^-/OH^-$ exchanger tributyltin, and different $Cl^-$ concentrations. Under these conditions $[Cl^-]_i$ follows $[Cl^-]_o$. Calibration procedures similar to that in Fig. 1 *a* yielded a Stern-Volmer constant (Kq) for SPQ in the cells of 17.3 $\pm$ 0.8 $M^{-1}$ ($n = 5$), which is close to that reported before with other cell types (Chao, Dix, Sellers, and Verkman, 1989; Chao, Widdicombe, and Verkman, 1990; Foskett, 1990). Previous studies showed that the Kq for SPQ in solution

is affected by pH and the type of buffer used (Vasseur, Frargne, and Alvarado, 1993). We therefore tested the effect of $pH_i$ on dye fluorescence. After clamping $pH_i$ at 7.2 and $[Cl^-]_i$ at 0, 20, or 100 mM with the ionophores/high $K^+$ medium, the cells were perfused with the same solutions buffered to pH of 7.6. Fluorescence intensity changed by <5% and only at 0 $[Cl^-]_i$ conditions (not shown). The sensitivity of SPQ and other $Cl^-$-sensitive dyes to carboxylate groups probably accounts for the minimal effect of pHi on the dye in the cells (Koncz and Dougidas, 1994). Using a Kq of 17.3 and measurement of total anion-dependent fluorescence, the $[Cl^-]_i$ of resting acinar cells was found to be 65.4 ± 4.2 mM ($n = 58$).

Fig. 1 *b* compares the rate of $Cl^-$ efflux and influx in the presence and absence of $HCO_3^-$. Exposing the cells to a $HCO_3^-$-buffered medium caused a transient increase in $[Cl^-]_i$. This was probably due to accumulation of intracellular $HCO_3^-$ and activa-
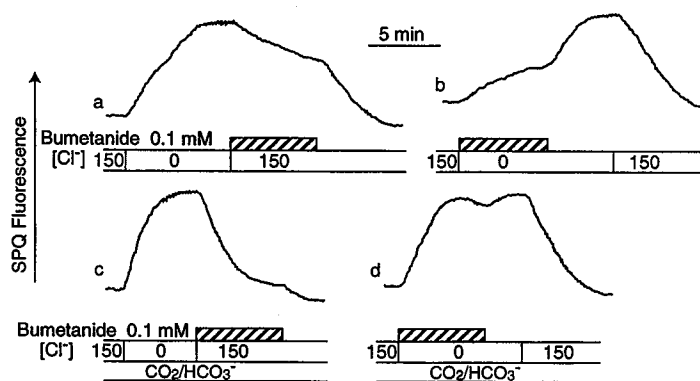


FIGURE 2. $Cl^-$ fluxes by the NaK2Cl cotransporter. In experiments *a* and *b*, SPQ-loaded acinar cells in solution A (NaCl) were perfused with $Cl^-$-free solution B. As indicated, solution A (*a*) or solution B (*b*) contained 0.1 mM bumetanide. For the experiments in *c* and *d*, cells in solution A (HEPES) were perfused with solution E ($HCO_3^-$) for at least 10 min before solution changes. All solutions in experiments *c* and *d* were buffered with $CO_2/HCO_3^-$. Where indicated, the cells were perfused with solutions E (NaCl) or F ($HCO_3^-$, $Cl^-$ free) and with or without 0.1 mM bumetanide.

tion of the $Cl^-/HCO_3^-$ exchanger. The $Cl^-/HCO_3^-$ exchanger of acinar cells transports $HCO_3^-$ but not $OH^-$ (Muallem and Loessberg, 1990a). Removal of external $Cl^-$ in the presence and absence of $HCO_3^-$ increased SPQ fluorescence to the same extent, indicating similar depletion of $Cl^-_i$. However, $HCO_3^-$ increased the rate of $Cl^-$ efflux from 8.83 ± 0.31 to 21.9 ± 0.96 mM/min and the rate of $Cl^-$ influx from 9.25 ± 0.43 to 20.2 ± 0.74 mM/min ($n = 12$). Again, this is likely due to activation of the $Cl^-/HCO_3^-$ exchanger by $HCO_3^-$.

Ion substitution and several inhibitors were used to identify the transporters mediating $Cl^-$ fluxes in the presence and absence of $HCO_3^-$. Fig. 2, *a* and *b*, shows that the NaK2Cl cotransport inhibitor bumetanide inhibited $Cl^-$ influx and efflux of cells bathed in HEPES-buffered medium (62 ± 8%, $n = 3$). In the presence of $HCO_3^-$, bumetanide had a relatively small effect on the rate or extent of $Cl^-$ fluxes. The results in Fig. 3 *a* provide further evidence for NaK2Cl contransport in acinar

cells by showing that removal of external $K^+$ was as effective as bumetanide in inhibiting $Cl^-$ influx.

The partial inhibition of $Cl^-$ fluxes by bumetanide and removal of external $K^+$ suggest the presence of additional $Cl^-$ transport mechanisms in acinar cells. Fig. 3, *b–d*, shows that some of these fluxes are probably mediated by NaCl cotransport. Removal of extracellular $Na^+$ almost completely inhibited $Cl^-$ influx into $Cl^-$-depleted cells (Fig. 3 *b*). In addition, the NaCl cotransport inhibitor thiazide (TZ) (Gamba, Miyanoshita, Lombardi, Lytton, Lee, Hediger, and Hebert, 1994) partially inhibited $Cl^-$ efflux (Fig. 3 *c*) and $Cl^-$ influx (Fig. 3 *d*) in acinar cells. Effects of TZ could be demonstrated in HEPES (Fig. 3) but not in $HCO_3^-$-buffered medium (not shown), due to the dominance of the $Cl^-/HCO_3^-$ exchanger in $Cl^-$ fluxes.
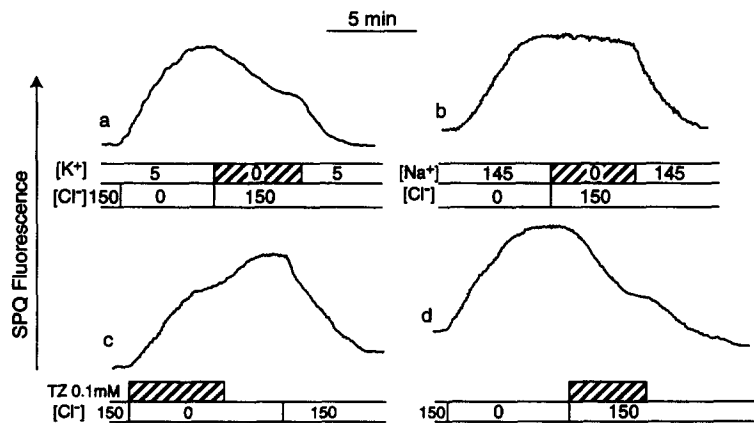


FIGURE 3.   NaCl cotransport in pancreatic acini. In experiment *a*, cells incubated in solution B ($Cl^-$ free) were exposed to solution D ($K^+$ free) and then to solution A to test the effect of $K_o^+$ on $Cl^-$ influx. In experiment *b*, cells incubated in solution B were exposed to solution C ($Na^+$ free) and then to solution A to test the effect of $Na_o^+$ on $Cl^-$ influx. In experiments *c* and *d*, the cells were perfused with solution A or B and in the presence or absence of 0.1 mM TZ as indicated.

## $Na^+$ Transport

The previous section showed the transport of $Cl^-$ by two $Na^+$-dependent mechanisms. To evaluate their relative role in $Na^+$ transport and the presence of other $Na^+$ transporters, intracellular $Na^+$ concentration ($[Na^+]_i$) was measured with the $Na^+$-sensitive fluorescent dye SBFI. Fig. 4 *a* shows an in situ calibration of the SBFI fluorescence ratio. The membrane was permeabilized to monovalent ions with the ionophores gramicidin and monensin. Under these conditions, changes of $Na_o^+$ resulted in rapid and stable changes in the fluorescence ratio. Assuming a 1:1 stoichiometry for the interaction of $Na^+$ with SBFI (Minta and Tsien, 1989; Harootunian, Kao, Eckert and Tsien, 1989), the dye in pancreatic acinar cells showed an apparent Kd for $Na^+$ of $\sim$39 $\pm$ 7 mM ($n = 4$). Using this Kd, $[Na^+]_i$ in resting pancreatic acinar cells was found to be 11.7 $\pm$ 0.5 mM ($n = 36$).

Fig. 4 $b$ shows that removal of $Na_o^+$ resulted in depletion of $Na_i^+$ and that addition of $Na_o^+$ caused $[Na^+]_i$ to increase to normal levels without a noticeable overshoot. $Na^+$ efflux and influx rates remained the same when this procedure was repeated up to three times. The membrane permeability to $Na^+$ was estimated from inhibition of the $Na^+$ pump with ouabain, which is the major $Na^+$ efflux mechanism in resting cells. Addition of 100 μM ouabain caused $[Na^+]_i$ to increase at an initial
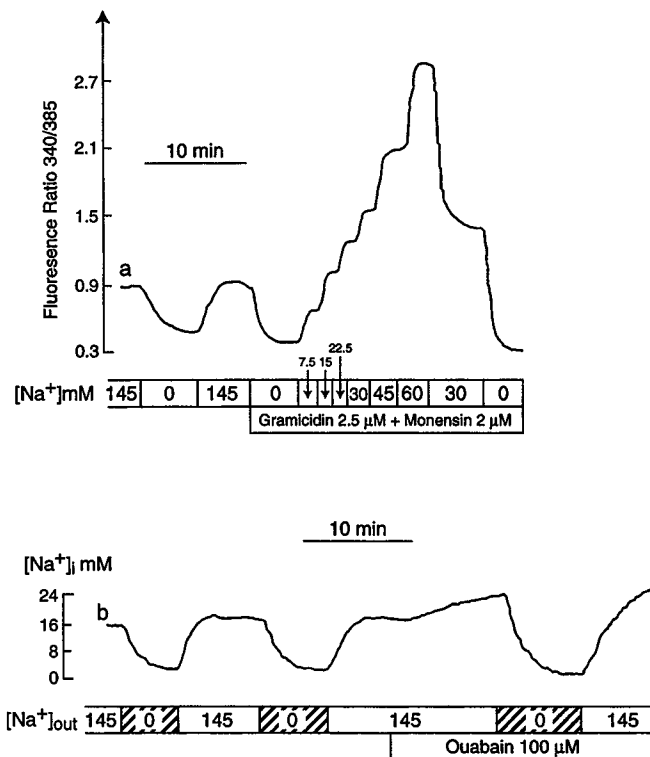


FIGURE 4.   Measurement of $[Na^+]_i$ in acinar cells. In experiment $a$, acinar cells loaded with SBFI were perfused with solution C ($Na^+$ free). Then the cells were incubated in solution C containing 2.5 μM gramicidin and 2 μM monensin, or mixtures of solution A and C to give the indicated $[Na^+]$, which also contained the ionophores. The $Na^+$-dependent change in the fluorescence ratio was recorded at excitation wavelengths of 340 and 385 nm. In experiment $b$, the cells were alternately perfused with relations A (NaCl) or C ($Na^+$ free). Where indicated, the perfusion medium contained 0.1 mM ouabain.

rate of ∼1.56 ± 0.11 mM/min, which was then gradually reduced to 0.68 ± 0.08 mM/min ($n = 6$). Similar results were obtained when the $Na^+$ pump was inhibited by removal of external $K^+$ (see Zhao and Muallem, 1995). The low $Na^+$ permeability of resting cells was maintained for many minutes. In fact, incubation of resting acinar cells with ouabain for 30, 60, and 120 min caused $[Na^+]_i$ to increase to 21 ± 4, 46 ± 6, and 102 ± 7 mM ($n = 3$), respectively. However, removal and addition of

$Na^+$ to the incubation medium of ouabain-treated acini resulted in relatively rapid $Na^+$ fluxes, but again without causing an overshoot (Fig. 4 $b$).

The most reproducible protocol to identify mechanisms of $Na^+$ transport in acinar cells was to measure $Na^+$ influx into cells incubated in the absence of $Na_o^+$. Fig. 5, $a$–$c$, shows that TZ and TEA had no measurable effect on $Na^+$ influx. The concentrations of TZ and TEA used were optimal in affecting ^{86}Rb uptake in resting and stimulated cells (see below). Increasing TZ up to 0.5 mM and TEA up to 20 mM had no further effect on $Na^+$ influx or ^{86}Rb uptake (not shown). On the other hand, bumetanide inhibited the rate and extent of $Na^+$ influx by ~32%, and removal of bumetanide resulted in recovery of normal $[Na^+]_i$ (Fig. 5 $d$). These findings were corroborated by testing the effect of external $K^+$ and $Cl^-$ on $Na^+$ influx. Fig. 6 shows that removal of external $K^+$ (Fig. 6 $b$, *top*) or external $Cl^-$ (Fig. 6 $d$, *bottom*) reduced the rate of $Na^+$ influx by ~37%. In the absence of external $K^+$, $[Na^+]_i$
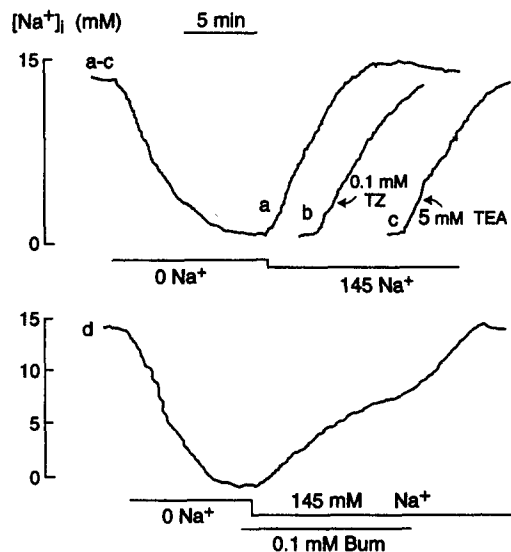


FIGURE 5. $Na^+$ influx by the NaK2Cl cotransporter. In all experiments, the cells were perfused with solution C ($Na^+$ free). After stabilization of $[Na^+]_i$, the cells were perfused with solutions C and then A (+$Na^+$) ($a$) also containing 0.1 mM TZ ($b$), 5 mM TEA ($c$), or 0.1 mM bumetanide. Where indicated in trace $d$, bumetanide was removed by perfusion with solution A.

did not stabilize at a reduced level, probably due to inhibition of the $Na^+$ pump (Fig. 6 $b$). In the absence of $Cl_o^-$ and the presence of $Na_o^+$, $[Na^+]_i$ stabilized below resting level and addition of $Cl_o^-$ caused a further increase in $[Na^+]_i$.

The results in Figs. 5 and 6 indicate that a small portion (30–35%) of $Na^+$ influx into acinar cells is mediated by the NaK2Cl cotransporter, whereas most of the influx is mediated by a $K_o^+$- and $Cl_o^-$-independent mechanism. This is likely to be the $Na^+/H^+$ exchanger. It was not possible to test directly the contribution of the exchanger to $Na^+$ influx using SBFI since amiloride and all analogues tested entered the cells and dominated the fluorescence. Another alternative was to use the new $Na^+$-sensitive fluorescent dye $Na^+$-Green. Fig. 7 shows the behavior of this dye in pancreatic acinar cells. In ~31% (29/94) of the experiments, the dye appeared to be located in the cells, probably in the cytoplasm (Fig. 7 $a$). Hence, removal and re-addition of $Na_o^+$ resulted in relatively small and slow changes in the fluorescence.

Exposing the cells to digitonin caused an initial increase in fluorescence, probably due to an increased permeability of the plasma membrane to $Na^+$ before permeabilization to $Na^+$-Green. Subsequently, the cells lost $\sim84 \pm 9\%$ ($n = 8$) of their fluorescence. On the other hand, in $\sim69\%$ (65/94) of the experiments, a significant amount of the dye (between 65 and 96%) was localized in a digitonin-insensitive space. This portion of the dye rapidly responded to changes in external $Na^+$ before and after digitonin treatment (Fig. 7 $b$). In addition, the changes in fluorescence due to removal and addition of $Na_o^+$ were not affected by any combination of $Na^+$ transport inhibitors or removal of $K^+$ and $Cl^-$ from the medium. Therefore, we believe most of the dye was localized in the external surface of the plasma membrane. We attempted to improve dye loading with little success. The parameters tested (between 2 to 3 experiments each) were the incubation temperature (20–37°C), loading time (1–60 min), dye concentration (0.5–10 μM), number of cells



[Na$^+$]$_i$ (mM)
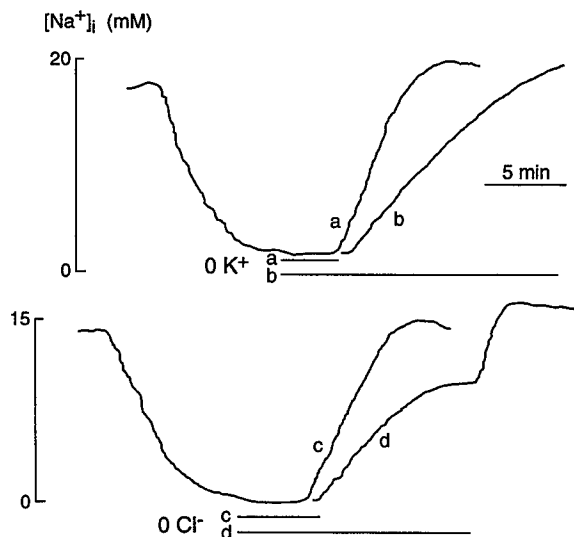
FIGURE 6. Effect of $K_o^+$ and $Cl_o^-$ on $Na^+$ influx. In experiments $a$ and $b$, acinar cells incubated in solution C were exposed to solution C in which 5 mM NMG$^+$Cl$^-$ replaced 5 mM KCl (K$^+$ free and Na$^+$ free) and then to solution A (trace $a$, control) or solution D (K$^+$ free, Na$^+$ containing) ($b$). In experiments $c$ and $d$, cells in solution C were exposed to solution C in which 145 mM NMG$^+$ Glu$^-$ replaced 145 mM NaCl and then to solution A ($c$) or solution B (Cl$^-$ free) and solution A ($d$).

(20–200 mg/ml), and structural units (single cells, small and large acinar clusters). At 37°C and at incubation times above 20 min, the dye behaved as if it was mostly extracellular. Loading freshly prepared cells at room temperature for 15 min resulted in a sufficient fluorescence signal to allow reliable measurement of [Na$^+$]$_i$ changes when the dye was mostly intracellular (31% of cells). The effect of the other parameters tested was not obvious. In a preparation where the dye was intracellular or extracellular, most of the cells in this preparation showed similar dye localization.

The advantage of using Na$^+$-Green is that the contribution of the Na$^+$/H$^+$ exchanger to Na$^+$ fluxes can be directly studied using amiloride analogues. This transporter has a prominent role in Na$^+$ homeostasis in resting and stimulated cells (see below) so that direct evaluation of its contribution is important. Fig. 7 $d$ shows that complete inhibition of the Na$^+$/H$^+$ exchanger with 25 μM dimethylamiloride

(DMA) reduced the rate of $Na^+$ influx by 67 ± 4.6% ($n$ = 11). Bumetanide together with DMA inhibited the influx by 86 ± 6.6% ($n$ = 5). Removal of the blockers resulted in recovery of resting $[Na^+]_i$. It was not possible to reliably calibrate the $Na^+$-Green signals because this dye does not have isosbestic point with respect to $Na^+$, which prevented correction for dye leak, photobleaching, and extracellular
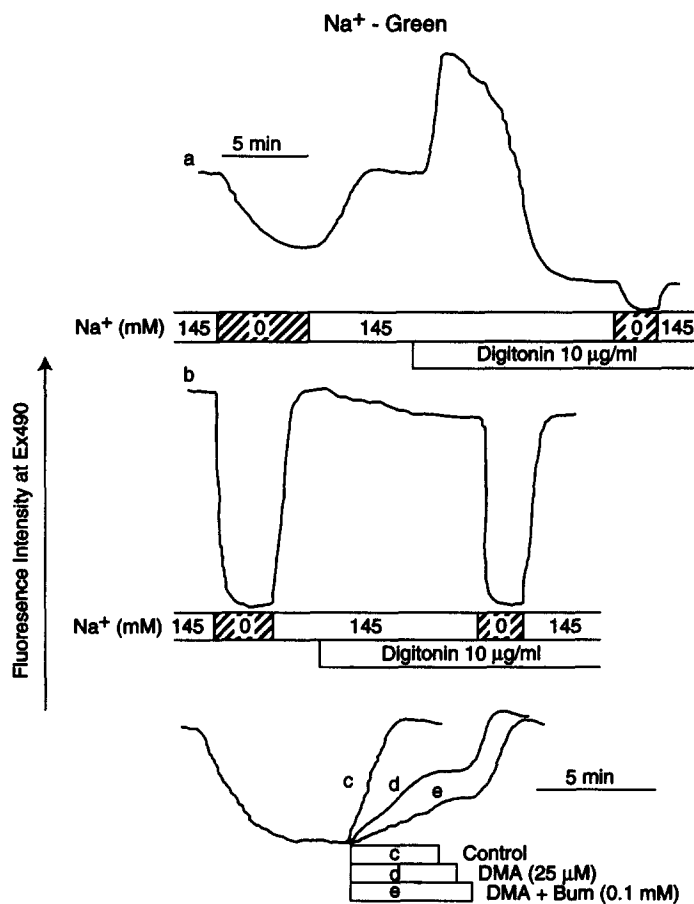


FIGURE 7.   Measurement of $[Na^+]_i$ with $Na^+$-Green. In experiments *a* and *b*, acinar cells loaded with $Na^+$-Green were alternately perfused with solution A (NaCl) or solution C ($Na^+$ free). Where indicated, the solutions contained 10 μg/ml digitonin to permeabilize the cells to the dye. In experiments *c-e*, cells loaded with intracellular $Na^+$-Green were incubated with solution C and then perfused with solution A (control, *c*) containing 25 μM DMA (*d*) or DMA and 0.1 mM bumetanide (*e*) and finally with solution A.

binding. Nonetheless, when most of the dye was intracellular, it seemed to accurately report the contribution of the $Na^+/H^+$ exchanger to $Na^+$ homeostasis.

The fluorescent probes report the net changes in the concentration of the relevant ions. To study the relationship between the transporters we had to measure their activity at steady state. To achieve that, we followed the unidirectional influx

of $K^+$ using $^{86}Rb$, which did not require a prior change of the steady state level of any ion. These results are summarized in Fig. 8. Preliminary experiments showed that $^{86}Rb$ uptake was linear for at least 6 min at 37°C. The first 4 min of the uptake is illustrated in Fig. 8. Inhibition of the $Na^+$ pump of resting cells with 0.5 mM ouabain reduced the rate of $^{86}Rb$ by ~14%. Similar results were obtained with 0.1 or up to 2 mM ouabain. Bumetanide inhibited the uptake by ~29%. However, the combination of ouabain and bumetanide inhibited the uptake by 97%. Hence, there was a large overlap between the ouabain-sensitive and bumetanide-sensitive $^{86}Rb$ uptake into pancreatic acinar cells.
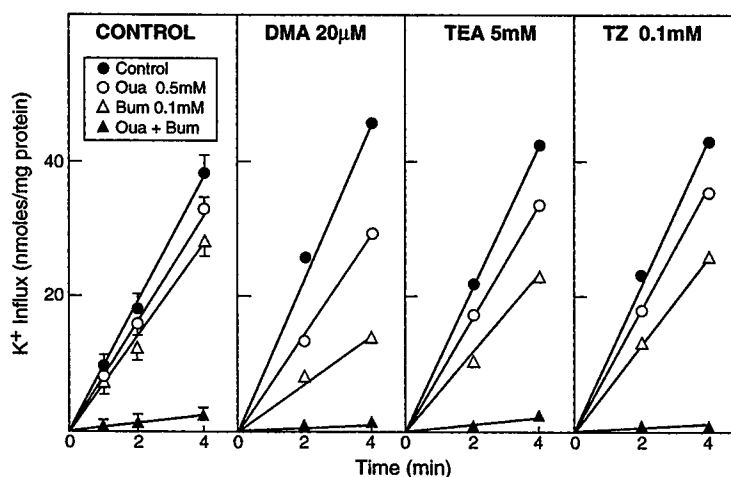


FIGURE 8.   Properties of $^{86}Rb$ influx into pancreatic acinar cells. Acinar cells in solution A were incubated for 20 min at 37°C before dilution into solution A containing $^{86}Rb$ and either no further additions (*solid circles*), 0.5 mM ouabain (*open circles*), 0.1 mM bumetanide (*open triangles*), or ouabain and bumetanide (*solid triangles*). In some experiments, the incubation solution also contained 20 μM DMA (*second column*), 5 mM TEA (*third column*), or 0.1 mM TZ (*fourth column*). At the indicated times, samples were removed to determine the cellular content of $^{86}Rb$, as described in Materials and Methods. All points are the mean ± SEM of three to four separate experiments performed in duplicate. The SEM are shown only for the experiments in the first column, but they were similar for all experiments.

Inhibition of the $Na^+/H^+$ exchanger with DMA enhanced, rather than reduced, $^{86}Rb$ uptake by 18.6 ± 3.4% ($n = 4$, $P < 0.05$). Furthermore, in the presence of DMA ouabain inhibited $^{86}Rb$ uptake by 33.4 ± 1.9% and bumetanide inhibited $^{86}Rb$ uptake by 66.9 ± 2.7%. Thus, in the presence of DMA, ouabain and, in particular, bumetanide alone were more effective in inhibiting $^{86}Rb$ uptake. A similar trend was observed with TEA and TZ. The increase in $Na^+$ uptake due to TEA or TZ was between 6 and 10% but did not reach statistical significance. Although ouabain appears more effective in the presence of either blocker, the effect was relatively small. However, both blockers significantly increased the fraction of bumet-

anide-sensitive $^{86}$Rb uptake (Fig. 8). In the presence of TEA bumetanide-sensitive uptake (21.3 nmol/mg protein/4 min) was about twice that in the absence of TEA and accounted for 49.7 ± 3.8% ($n$ = 3) of the uptake. In the presence of TZ, bumetanide inhibited the uptake by 40.4 ± 4.2% ($n$ = 3).

### DISCUSSION

In addition to digestive enzymes, pancreatic acinar cells secret isotonic, plasma-like fluid (Schulz, 1987; Case, 1989). This requires the vectorial, transepithelial transport of $Na^+$ and $Cl^-$. Most of the $Cl^-$ transport is transcellular, whereas $\sim$70% of $Na^+$ is transported through a paracellular pathway (Kuijpers and dePont, 1987; Case, 1989). It is generally accepted that $Cl^-$ exits the AM through $Ca^{2+}$-activated $Cl^-$ channels (Petersen and Findlay, 1987; Petersen, 1992). However, the $Cl^-$ entry pathway(s) at the basolateral membrane is not known. To identify the dominant pathway of $Cl^-$ entry during stimulation of fluid secretion, it was necessary to characterize the properties of $Cl^-$ transport in acinar cells. $Cl^-$ transport is directly or indirectly coupled to the transport of $Na^+$ and $K^+$. In fact, a tight coupling between $Na^+$ and $Cl^-$ influx into stimulated acinar cells has been reported (O'Doherty and Stark, 1983). Therefore, it was also necessary to examine the role of $Na^+$ and $K^+$ in $Cl^-$ transport and the relationship between the various transporters controlling the cytosolic concentrations of $Cl^-$, $Na^+$, and $K^+$. Indeed, the overall conclusion of the present studies is that the various transporters appear to be sensitive to the concentration of intracellular $K^+$ and $Na^+$, and electrolyte secretion is regulated through modulation of $[Na^+]_i$.

### $Cl^-$ Transport

Measurements with SPQ showed that in resting acinar cells, $[Cl^-]_i$ was 65.4 mM, which is approximately fourfold above equilibrium. This value is close to the 68.9 mM reported in mouse acinar cells using $Cl^-$ selective microelectrodes (O'Doherty and Start, 1983). Several transporters were found to support the uphill transport of $Cl^-$ in acinar cells. The first is a NaK2Cl cotransport system. Previous studies reported the presence of a furosemide-sensitive $K^+$ and $^{86}$Rb efflux in mouse pancreatic acini (Singh, 1984; Petersen and Singh, 1985). Here we show that in the absence of $HCO_3^-$ this transporter mediates $\sim$70% of $Cl^-$ and 30% of $Na^+$ uptake into the cells. Thus, $Cl^-$ influx and efflux could be partially inhibited by bumetanide or removal of external $K^+$ or $Na^+$, and $Na^+$ influx was partially inhibited by bumetanide and removal of external $K^+$ or $Cl^-$. Another $Cl^-$ transporter not described before in pancreatic acini is the NaCl cotransporter. TZ is a relatively specific inhibitor of this transporter (Gamba et al., 1994). TZ reduced $Cl^-$ influx and efflux in acinar cells. Removal of $K_o^+$ inhibited, but did not prevent, $Cl^-$ influx into $Cl^-$-depleted acinar cells. Only in the absence of $Na_o^+$ (and in the presence or absence of $K_o^+$) was $Cl^-$ influx largely inhibited. This also indicates that in the absence of $HCO_3^-$, the $Na^+$-dependent cotransporters mediate most of $Cl^-$ fluxes in acinar cells.

A different behavior was found in the presence of $HCO_3^-$. Under these conditions, the $Cl^-/HCO_3^-$ exchanger dominated the $Cl^-$ fluxes. Not only did $HCO_3^-$

more than double the rates of $Cl^-$ influx and efflux, but bumetanide had minimal effect (Fig. 2), whereas TZ and TEA had no apparent effect (not shown) on $Cl^-$ fluxes. By following $pH_i$, we have previously shown the presence of a $Cl^-/HCO_3^-$ exchanger in acinar cells that transports $HCO_3^-$ but not $OH^-$ (Muallem and Loessberg, 1990a). The measurement of $[Cl^-]_i$ provides the first evidence that the $Cl^-/HCO_3^-$ exchanger dominates $Cl^-$ fluxes in acinar cells. An important implication of these findings is that the mechanism of fluid and electrolyte secretion by pancreatic acini is fundamentally different from that of the various salivary acinar cells (Petersen, 1992). In salivary acinar cells, the NaK2Cl cotransporter is the major $Cl^-$ influx pathway in the BLM (Foskett, 1990; Robertson and Foskett, 1994) and plays a central role in electrolyte secretion by these cells (Young, Cook, van Lennep, and Roberts, 1987; Petersen, 1992). Our findings indicate that in the presence of $HCO_3^-$, the cotransporter had a minimal role in transcellular $Cl^-$ transport in pancreatic acinar cells and that most of the $Cl^-$ entry across the BLM is mediated by the $Cl^-/HCO_3^-$ exchanger. Indeed furosemide had minimal effect (Seow et al., 1986) or no effect (Ishikawa and Kanno, 1991) on fluid and electrolyte secretion by pancreatic acinar cells. Continuous functioning of the $Cl^-/HCO_3^-$ exchanger during electrolyte secretion requires the parallel activation of the $Na^+/H^+$ exchanger to maintain stable $pH_i$. Activation of the $Na^+/H^+$ exchanger can also fuel the $Na^+$ pump. Measurements of $[Na^+]_i$ confirmed the coupling between the $Na^+/H^+$ exchanges and the $Na^+$ pump.

*Na⁺ Transport*

Measurements with SBFI showed that in resting acinar cells, $[Na^+]_i$ was $\sim11.7$ mM. Previous measurements with $Na^+$-selective microelectrodes reported a resting $[Na^+]_i$ of between 8.3 and 10.5 mM (O'Doherty and Stark, 1982, 1983). The $Na^+$ permeability of resting cells was low, as evident from the rate of $[Na^+]_i$ increase after inhibition of the $Na^+$ pump with ouabain or removal of $K_o^+$. Therefore, to identify the transporters mediating $Na^+$ influx, it was necessary to deplete the cells from $Na^+$. In addition to the NaK2Cl and the NaCl cotransporters, a voltage-independent, nonselective cation channel (Maruyama and Petersen, 1982; Petersen, 1992) and a $Na^+/H^+$ exchange mechanism (Muallem and Loessberg, 1990a) can mediate $Na^+$ influx into acinar cells. The present studies show that in the presence of $Cl^-$, the NaCl cotransporter and a TEA-sensitive channel had minimal effect on $Na^+$ influx into $Na^+$-depleted cells. A significant part of the influx was dependent on the presence of $K_o^+$ and $Cl_o^-$, and inhibited by bumetanide, suggesting that the NaK2Cl cotransporter mediates some of the $Na^+$ influx into $Na^+$-depleted cells, even when the cells contained $Cl^-$. However, most of the $Na^+$ influx (65–70%) was sensitive to amiloride and therefore mediated by the $Na^+/H^+$ exchanger.

In resting cells, all the $Na^+$ transporters operated at a fraction of their maximal capacity. This is evident from the low $Na^+$ permeability of the cells and the small effect of the various blockers on $[Na^+]_i$. This is despite an inwardly directed driving force for the NaCl and NaK2Cl cotransporters. It is well established that the $Na^+/H^+$ exchanger is sensitive to $pH_i$ (Aronson, Nee, and Suhm, 1982; Grinstein and Foskett, 1990), with minimal activity at physiological $pH_i$. It is likely that the other transporters are also regulated to maintain low activity in resting cells. Recently we

have shown that the NaK2Cl cotransporter is sensitive to $[Na^+]_i$ (Whisenant, Khademazad, and Muallem, 1993). Similar findings were reported in ciliary epithelial cells (Dong, Delamere, and Coca-Prados, 1994). In parotid acinar cells, all major $Na^+$ influx transporters appear to be sensitive to $[Cl^-]_i$ (Robertson and Foskett, 1994). A combination of these regulatory mechanisms is likely to minimize the activity of the $Na^+$ influx transporters in resting acinar cells.

### Coupling of Transporters

Measurement of $^{86}Rb$ fluxes revealed an intimate relationship between the various transporters (Fig. 8). The model in the accompanying manuscript (Zhao and Muallem, 1995) illustrates these findings. All the $Na^+$ transporters feed the $Na^+$ pump to fuel $Na^+$ efflux and $K^+$ influx. Because the transport of $Na^+$ is coupled to other ions, the transporters also respond to changes in the concentrations of these ions. For example, the NaCl cotransporter responds to the internal concentration of $Na^+$ and $Cl^-$ and the NaK2Cl cotransporter to the intracellular concentrations of all ions. The first of these relationships is noted when neither bumetanide nor ouabain alone can inhibit $^{86}Rb$ influx, whereas in the presence of both blockers, $K^+$ influx is completely blocked. Obviously, when the NaK2Cl cotransporter is inhibited, another transporter is mediating $Na^+$ influx into the cells to fuel the $Na^+$ pump. This transporter appears to be the $Na^+/H^+$ exchanger (Fig. 8). When the $Na^+$ pump is blocked, the NaK2Cl cotransport activity is increased (compare the bumetanide sensitive uptake in the presence and absence of ouabain). Hence, it seems that the activities of the cotransporter and the $Na^+$ pump are modified to complement each other with respect to $K^+$ influx. In resting cells, the coupling is unlikely to be through $[Na^+]_i$, since inhibition of $Na^+$ influx by the cotransporter should reduce $Na^+$ pump activity, and an increase in $[Na^+]_i$ due to inhibition of the $Na^+$ pump is expected to inhibit net $Na^+$ uptake by the cotransporter (Whisenant et al., 1993). Thus, it is likely that in resting acinar cells, the $Na^+/K^+$ pump and the NaK2Cl cotransporter are coupled through $[K^+]_i$. In this case the two transporters should be sufficiently sensitive to detect small changes in $[K^+]_i$ in the presence of 140–145 mM $K^+$. One way to achieve that would be if the transporters are localized next to each other to affect the local concentration of $K^+$. The influence of $[K^+]_i$ on NaK2Cl cotransport and the $Na^+$ pump is not well understood, although effects of $[K^+]_i$ on partial reactions mediated by the two transporters have been described (Glynn, 1985; Lauf, McManus, Haas, Forbush, Duhm, Flatman, Saier, and Russell, 1987).

A major form of coupling appears to be through the supply of $Na^+$ to the $Na^+$ pump. Inhibition of $Na^+$ influx through the nonselective channel (TEA), the NaCl cotransporter (TZ) or the $Na^+/H^+$ exchanger (DMA) increased the total but, in particular, the bumetanide-sensitive $^{86}Rb$ uptake. Since changes in coupling stoichiometry of the $Na^+$ pump by the blockers are not likely, the increased $^{86}Rb$ uptake indicates that the activity of the NaK2Cl cotransporter is increased to compensate for the reduction in $Na^+$ influx. Since $Na^+$ influx is now coupled to $K^+$ influx, the total and bumetanide-sensitive fluxes are increased. The overall effect is increased coupling between the activities of the $Na^+$ pump and the NaK2Cl cotransporter. In addition, these findings show that the relative contribution of the different trans-

porters to $Na^+$ influx can be evaluated from their effect on bumetanide-sensitive $^{86}Rb$ uptake. Thus, the $Na^+/H^+$ exchanger contributes twice more than the cotransporter and three times more than the NaCl cotransporter and the TEA-sensitive channel to $Na^+$ influx in resting cells (Fig. 8; Zhao and Maullem, 1995).

Coupling among the transporters through the concentrations of $Na^+_i$ and $K^+_i$ indicates that most of the transporters are likely to be regulated by these ions through interaction with a cytosolic domain. It is also likely that agonists that stimulate electrolyte secretion by pancreatic acinar (and probably other) cells do so by modification of $Na^+_i$ and/or $K^+_i$. The companion article (Zhao and Muallem, 1995) describes how pancreatic secretagogues regulate the activity of specific transporters to modulate $[Na^+]_i$ and the overall process of fluid and electrolyte secretion by acinar cells.

REFERENCES

Aronson, P. S., J. Nee, and M. A. Suhm. 1982. Modifier role of internal $H^+$ in activating the $Na^+/H^+$ exchanger in renal microvillus membrane vesicles. *Nature.* 299:161–163.

Case, M. R. 1989. Physiology and biochemistry of pancreatic exocrine secretion. *Current Opinions in Gastroenterology.* 5:665–681.

Case, M. R., and B. E. Argent. 1986. Bicarbonate secretion by pancreatic duct cells—mechanisms and control. *In* The Exocrine Pancreas: Biology, Pathology and Diseases. V. L. Go et al., editors. Raven Press, NY. 213–242.

Chao, A. C., J. A. Dix, M. C. Sellers, and A. S. Verkman. 1989. Fluorescence measurement of chloride transport in monolayer cultured cells. Mechanisms of chloride transport in fibroblasts. *Biophysical Journal.* 56:1071–1081.

Chao, A. C., J. H. Widdicombe, and A. S. Verkman. 1990. Chloride conductive and cotransport mechanisms in cultures of canine tracheal epithelial cells measured by an entrapped fluorescent indicator. *Journal of Membrane Biology.* 113:193–202.

Dong, J., N. A. Delamere, and M. Coca-Prados. 1994. Inhibition of $Na^+$-$K^+$-ATPase activates $Na^+$-$K^+$-$2Cl^-$ cotransporter activity in cultured ciliary epithelium. *American Journal of Physiology.* 266:C198–C205.

Dufresne, M., M.-J. Bastie, N. Vaysse, Y. Creach, E. Hollande, and A. Ribet. 1985. The amiloride sensitive $Na^+/H^+$ antiport in guinea pig pancreatic acini. *FEBS Letters.* 187:126–130.

Evans, L. A., D. Pirani, D. I. Cook, and J. A. Young. 1986. Intraepithelial current flow in rat pancreatic secretory epithelia. *Pflugers Archives.* 407(Suppl. 2):S107–S111.

Foskett, J. K. 1990. $[Ca^{2+}]_i$ modulation of $Cl^-$ content controls cell volume in single salivary acinar cells during fluid secretion. *American Journal of Physiology.* 259:C998–C1004.

Gamba, G., A. Miyanoshita, M. Lombardi, J. Lytton, W.-S. Lee, M. A. Hediger, and S. C. Hebert. 1994. Molecular cloning, primary structure, and characterization of two members of the mammalian electroneutral sodium (potassium)-chloride cotransporter family expressed in kidney. *Journal of Biological Chemistry.* 269:17713–17722.

Gardner, J. D., and R. T. Jensen. 1986. Receptors and cell activation associated with pancreatic en-

zyme secretion. *Annual Review of Physiology.* 48:103–117.

Glynn, I. M. 1985. The Na$^+$, K$^+$-Transporting Adenosine Triphosphatase. *In* The Enzymes of Biological Membranes. 2nd ed. A. N. Martonosi, editor. Plenum Press, New York. 3:35–114.

Grinstein, S., and J. K. Foskett. 1990. Ionic mechanism of cell volume regulation in leukocytes. *Annual Review of Physiology.* 52:399–414.

Harootunian, A. T., J. P. Y. Kao, B. K. Eckert, and R. Y. Tsien. 1989. Fluorescence ratio imaging of cytosolic free Na$^+$ in individual fibroblasts and lymphocytes. *Journal of Biological Chemistry.* 264:19458–19467.

Hootman, S. R., and J. A. Williams. 1987. Stimulus-secretion coupling in the pancreatic acinus. *In* Physiology of the Gastrointestinal Tract. L. R. Johnson, editor. Raven Press, New York. 1129–1146.

Ishikawa, T., and T. Kanno. 1988. Ionic basis of resting fluid secretion in the isolated perfused hypertrophied rat pancreas. *Biomedical Research.* 9:287–304.

Ishikawa, T., and T. Kanno. 1989. CCK-8–induced fluid secretion in the isolated and perfused hypertrophied rat pancreas. *Biomedical Research.* 10:49–64.

Ishikawa, T., and T. Kanno. 1991. Potassium transport across basolateral membrane of acinar cells in the perfused rat pancreas. *American Journal of Physiology.* 261:G570–G577.

Kanno, T., and M. Yamamoto. 1977. Differentiation between the calcium-dependent effects of cholecystokinin-pancreaozymin and the bicarbonate-dependent effects of secretin in exocrine secretion of the rat pancreas. *Journal of Physiology.* 264:787–799.

Koncz, C., and J. T. Dougidas. 1994. Use of MQAE for measurement of intracellular [Cl$^-$] in cultured aortic smooth muscle cells. *American Journal of Physiology.* 267:H2114–H2123.

Kuijpers, G. A. J., and J. J. H. H. M. dePont. 1987. Role of proton and bicarbonate transport in pancreatic cell function. *Annual Review of Physiology.* 49:87–103.

Lauf, P. K., T. J. McManus, M. Haas, B. Forbush, III, J. Duhm, P. W. Flatman, M. H. Saier, Jr., and J. M. Russell. 1987. Physiology and biophysics of chloride and cation cotransport across cell membranes. *Federation Proceedings.* 46:2377–2394.

Maruyama, Y., and O. H. Petersen. 1982. Single-channel currents in isolated patches of plasma membrane from basal surface of pancreatic acini. *Nature.* 299:159–161.

Minta, A., and R. Y. Tsein. 1989. Fluorescent indicators for cytosolic sodium. *Journal of Biological Chemistry.* 264:19449–19457.

Muallem, S. 1992. The ins and outs of Ca$^{2+}$ in exocrine cells. In Advances in Second Messenger and Phosphoprotein Research: Inositol Phosphates and Calcium Signaling. J. W. Putney, Jr., editor. Raven Press, New York. 351–368.

Muallem, S., and P. A. Loessberg. 1990a. Intracellular pH-regulatory mechanism in pancreatic acinar cells: I. Characterization of H$^+$ and HCO$\bar{3}$ transporters. *Journal of Biological Chemistry.* 265:12806–12812.

Muallem, S., and P. A. Loessberg. 1990b. Intracellular pH-regulatory mechanisms in pancreatic acinar cells: II. Regulation of H$^+$ and HCO$\bar{3}$ transporters by Ca$^{2+}$ mobilizing agonists. *Journal of Biological Chemistry.* 265:12813–12819.

O'Doherty, J., and R. J. Stark. 1982. Stimulation of pancreatic acinar secretion: increases in cytosolic calcium and sodium. *American Journal of Physiology.* 242:G513–G521.

O'Doherty, J., and R. J. Stark. 1983. A transcellular route for Na-coupled Cl transport in secreting pancreatic acinar cells. *American Journal of Physiology.* 245:G499–G503.

Petersen, O. H. 1992. Stimulus-secretion coupling: cytoplasmic calcium signals and the control of ion channels in exocrine acinar cells. *Journal of Physiology.* 448:1–51.

Petersen, O. H., and I. Findlay. 1987. Electrophysiology of the pancreas. *Physiological Review.* 67:1054–1116.

Petersen, O. H., and J. Singh. 1985. Acetylcholine evoked potassium release in the mouse pancreas.

*Journal of Physiology* (London). 365:319–329.

Petersen, O. H., and N. Ueda. 1977. Secretion of fluid and amylase in the perfused rat pancreas. *Journal of Physiology*. 264:819–835.

Robertson, M. A., and K. J. Foskett. 1994. Na$^+$ transport pathways in secretory acinar cells: membrane crosstalk mediated by [Cl$^-$]$_i$. *American Journal of Physiology*. 267:C146–C156.

Schulz, I. 1987. Electrolyte and fluid secretion in the exocrine pancreas. *In* Physiology of the Gastrointestinal Tract. L. R. Johnson, editor. Raven Press, New York. 1147–1171.

Seow, K. T. F., J. M. Lingard, and J. A. Young. 1986. Anionic basis of fluid secretion by rat pancreatic acini in vitro. *American Journal of Physiology*. 250:G140–G148.

Singh, J. 1984. Effects of acetylcholine and caerulein on $^{86}$Rb$^+$ efflux in the mouse pancreas: evidence for a sodium-potassium-chloride cotransport system. *Biochimica Biophysical Acta*. 775:77–85.

Thorn, P., and O. H. Petersen. 1992. Activation of nonselective cation channels by physiological cholecystokinin concentrations in mouse pancreatic acinar cells. *Journal of General Physiology*. 100:11–25.

Vasseur, M., R. Frargne, and F. Alvarado. 1993. Buffer-dependent pH sensitivity of the fluorescent chloride-indicator dye SPQ. *American Journal of Physiology*. 264:C27–C31.

Whisenant, N., M. Khademazad, and S. Muallem. 1993. Regulatory interaction of ATP, Na$^+$ and Cl$^-$ in the turnover cycle of the NaK2Cl cotransporter. *Journal of General Physiology*. 101:889–908.

Williams, J. A., and G. T. Blevins, Jr. 1993. Cholecystokinin and regulation of pancreatic acinar cell function. *Physiological Review*. 73:701–723.

Young, J. A., D. I. Cook, E. W. van Lennep, and M. L. Roberts. 1987. Secretion by the major salivary glands. *In* Physiology of the Gastrointestinal Tract. L. Johnson, J. Christensen, M. Jackson, E. Jacobson, and J. Walsh, editors. Raven Press, New York. 773–815.

Zhang, B.-X., J. Tortorici, X. Xu, and S. Muallem. 1994. Antagonists inactivate the IP$_3$-dependent Ca$^{2+}$ channel independent of IP$_3$ metabolism. *Journal of Biological Chemistry*. 269:17132–17135.

Zhao, H., and S. Muallem. 1995. Agonist-specific regulation of [Na$^+$]$_i$ in pancreatic acinar cells. *Journal of General Physiology*. 106:1243–1263.

Zhao, H., R. A. Star, and S. Muallem. 1994. Membrane localization of H$^+$ and HCO$_3^-$ transporters in the rat pancreatic duct. *Journal of General Physiology*. 104:57–85.