Agonist-specific Regulation of $[Na^+]_i$ in Pancreatic Acinar Cells

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ABSTRACT In a companion paper (Zhao, H., and S. Muallem. 1995), we describe the relationship between the major Na^+, K^+, and Cl^- transporters in resting pancreatic acinar cells. The present study evaluated the role of the different transporters in regulating $[Na^+]_i$ and electrolyte secretion during agonist stimulation. Cell stimulation increased $[Na^+]_i$ and ^{86}Rb influx in an agonist-specific manner. Ca^{2+}-mobilizing agonists, such as carbachol and cholecystokinin, activated Na^+ influx by a tetraethylammonium-sensitive channel and the Na^+/H^+ exchanger to rapidly increase $[Na^+]_i$ from ~11.7 mM to between 34 and 39 mM. As a consequence, the NaK2Cl cotransporter was largely inhibited and the activity of the Na^+ pump increased to mediate most of the $^{86}Rb(K^+)$ uptake into the cells. Secretin, which increases cAMP, activated the NaK2Cl cotransporter and the Na^+/H^+ exchanger to slowly increase $[Na^+]_i$ from ~11.7 mM to an average of 24.6 mM. Accordingly, secretin increased total ^{86}Rb uptake more than the Ca^{2+}-mobilizing agonists and the apparent coupling between the NaK2Cl cotransport and the Na^+ pump. All the effects of secretin could be attributed to an increase in cAMP, since forskolin affected $[Na^+]_i$ and ^{86}Rb fluxes similar to secretin. The signaling pathways mediating the effects of the Ca^{2+}-mobilizing agonists were less clear. Although an increase in $[Ca^{2+}]_i$ was required, it was not sufficient to account for the effect of the agonists. Activation of protein kinase C stimulated the NaK2Cl cotransporter to increase $[Na^+]_i$ and ^{86}Rb fluxes without preventing the inhibition of the cotransporter by Ca^{2+}-mobilizing agonists. The effects of the agonists were not mediated by changes in cell volume, since cell swelling and shrinkage did not reproduce the effect of the agonists on $[Na^+]_i$ and ^{86}Rb fluxes. The overall findings of the relationships between the various Na^+, K^+, and Cl^- transporters in resting and stimulated pancreatic acinar cells are discussed in terms of possible models of fluid and electrolyte secretion by these cells.

INTRODUCTION

Transepithelial electrolyte and fluid secretion by the stimulated pancreas are poorly understood on the cellular level (Case, 1989; Petersen, 1992). Spectrofluo-

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rometric and electrophysiologic studies revealed the presence of Na^+/H^+ (Dufresne, Bastie, Vaysse, Creach, Hollande, and Ribet, 1985; Muallem and Loessberg, 1990*a*) and Cl^-/HCO_3^- exchangers (Muallem and Loessberg, 1990*a*), K^+ and nonselective cation channels in the basolateral membrane (BLM) (Maruyama and Petersen, 1982; Thorn and Petersen, 1992) and Ca^{2+}-activated Cl^- channels in the apical membrane (AM) (Petersen, 1992). In a companion paper (Zhao and Muallem, 1995), we reported the measurements of $[Cl^-]_i$, $[Na^+]_i$, and ^{86}Rb fluxes to identify the major transporters regulating $[Cl^-]_i$, $[Na^+]_i$, and $[K^+]_i$ in resting acinar cells and the relationship between them. Three separate transporters mediated Cl^- and Na^+ fluxes in acinar cells; however, the Cl^-/HCO_3^- exchanger dominated $[Cl^-]_i$ regulation, whereas the Na^+/H^+ exchanger dominated $[Na^+]_i$ regulation (Zhao and Muallem, 1995).

Direct and indirect regulation of some of the transporters has been reported. For example, an increase in $[Ca^{2+}]_i$ closely correlates with activation of Cl^- channels believed to reside in the AM (Petersen, 1992). Stimulation of acinar cells with the Ca^{2+}-mobilizing agonists carbachol or CCK (Dufresne et al., 1985; Muallem and Loessberg, 1990*b*), but not the cAMP generating agonist secretin, were reported to stimulate the Na^+/H^+ exchanger (Bastie, Delvaux, Dufresne, Saunier-Blache, Vaysse, and Ribet, 1988) and the nonselective cation-permeable channel in the BLM (Thorn and Petersen, 1992). Ouabain binding and ^{86}Rb uptake in guinea pig acini demonstrated stimulation of the Na^+ pump by various agonists (Hootman, Ernest, and Williams, 1983; Hootman, Ochs, and Williams, 1985). The effects of the agonists were attributed to specific protein kinases, since an increase in cAMP and stimulation of protein kinase C (PKC) stimulated the ouabain-sensitive ^{86}Rb uptake (Hootman et al., 1985; Hootman, Brown, and Williams, 1987). However, it is not clear whether stimulation of the Na^+ pump by the agonist and second messengers is direct, or indirect through changes in $[Na^+]_i$. Furthermore, the relationship between the various monovalent ion transporters during stimulation of pancreatic acini (and most other cell types) is not known.

Building on the findings presented in our companion paper, here we used the same techniques and cellular preparation to study the regulation of the transporters by Ca^{2+}-mobilizing agonists and secretin, which increase cellular cAMP levels. The results indicate that pancreatic secretagogues regulate fluid and electrolyte secretion through modification of $[Na^+]_i$. Furthermore, the mechanism and extent of changes in $[Na^+]_i$ is agonist specific and determines the mode of coupling among monovalent ion transporters.

METHODS

All materials and experimental procedures were the same as in our companion paper (Zhao and Muallem, 1995), including composition of solutions, cell preparation, dye loading, and measurements of $[Na^+]_i$ $[Ca^+]_i$, and ^{86}Rb fluxes. The major incubation solution in most experiments was labeled solution A and contained (in mM) NaCl, 145; KCl, 5; $MgCl_2$ 1; $CaCl_2$, 1; HEPES, 10; and glucose, 10. When the effect of pretreatment with thapsigargin (Tg) or tetradecanoyl-phorbol-acetate (TPA) on ^{86}Rb uptake was measured, they were added during the last 2 or 6 min of the initial 20-min incubation at 37°C.

RESULTS

Na^+ Homeostasis in Stimulated Cells

When stimulated to secrete digestive enzymes, acinar cells also elaborate a small volume of fluid rich in NaCl and regulate their volume. To begin to explore the mechanism and ion transporters involved in these activities, we measured the effect of agonists on Na^+ homeostasis and the relationship between the transporters. Fig. 1 shows that stimulation of acinar cells with the Ca^{2+}-mobilizing agonist carbachol increased $[Na^+]_i$ in a concentration-dependent manner. In addition, increasing agonist concentration decreased the delay and increased the rate and extent of the

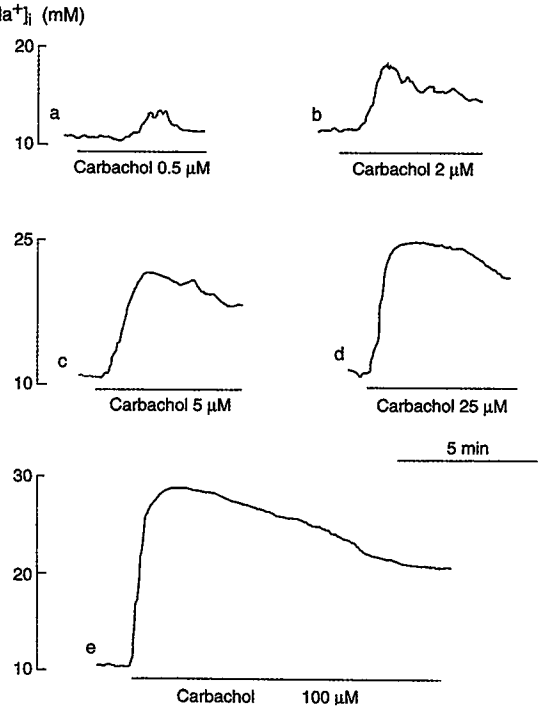

FIGURE 1. Effect of carbachol on $[Na^+]_i$. Acinar cells loaded with SBFI were stimulated with the indicated concentrations of carbachol. In these experiments the fluorescence was recorded from single cells within acini composed of 10–15 cells. Similar results were obtained when the fluorescence was recorded from 3–5 cells.

$[Na^+]_i$ increase. At maximal concentration (0.1 mM and above), carbachol increased $[Na^+]_i$ to about 34.3 ± 1.8 mM (n = 14) within 1 min of stimulation. We did not detect $[Na^+]_i$ oscillations at low agonist concentrations similar to those reported in parotid acinar cells (Wong and Foskett, 1991), although in parallel experiments carbachol at 0.2–5 μM did induce $[Ca^+]_i$ oscillations (not shown).

Fig. 2 demonstrates the effect of another Ca^{2+}-mobilizing agonist, CCK8, on $[Na^+]_i$ and compares it with the effect of secretin, which increases cellular cAMP levels. As with carbachol, stimulation with CCK8 caused a rapid $[Na^+]_i$ increase from 11 ± 0.8 mM to 39.1 ± 2.2 mM, which was then reduced and stabilized at 24 ± 3.7 mM (n = 6). To estimate the membrane permeability to Na^+ after stabili-

zation of $[Na^+]_i$, the cells were exposed to a Na^+-free solution, which resulted in depletion of Na^+_i. Addition of Na^+ to the perfusion medium caused an increase in $[Na^+]_i$ at a rate of 12.8 ± 3.1 mM/min, which was about threefold faster than that in resting cells (Zhao and Muallem, 1995). Stimulation of acinar cells with secretin caused a much slower increase in $[Na^+]_i$ (Fig. 2 *b*) with a maximum of 24.6 ± 3.5 mM ($n = 5$).

Blockers and ion substitution were used to determine the contribution of the different Na^+ influx pathways to the agonist-induced increase in $[Na^+]_i$. Fig. 3, *a* and *b* as well as *e* and *f*, shows that thiazide (TZ) and bumetanide had minimal effect on $[Na^+]_i$ when added before or during cell stimulation. A small reproducible effect

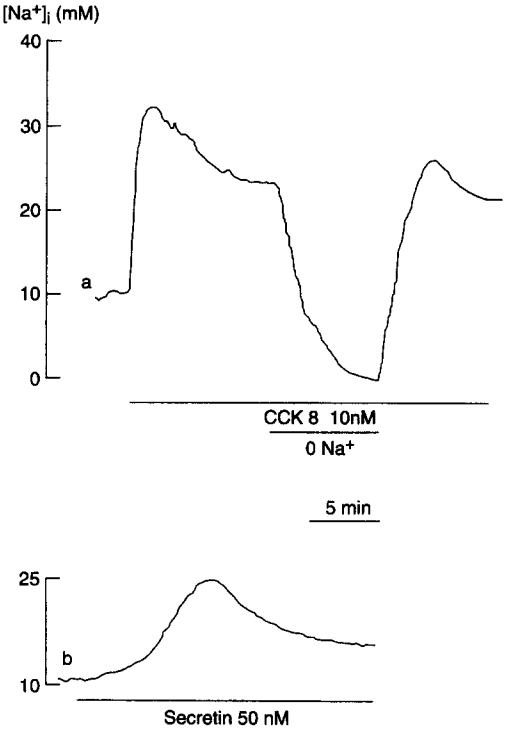

FIGURE 2. The effect of CCK8 or secretin on $[Na^+]_i$. Acinar cells loaded with SBFI were stimulated with the indicated concentrations of CCK8 (*a*) or secretin (*b*). In *a*, the cells were alternately perfused with solution A (NaCl) or solution C (Na^+-free).

of bumetanide ($n = 4$) could be observed using the protocol of Fig. 3 *f*. However, this effect was not different from that seen in Fig. 3 *e* before cell stimulation. A more prominent effect was recorded with TEA, which inhibits K^+ channels and cation-specific, nonselective channels in acinar cells. Addition of TEA before cell stimulation reduced the agonist-dependent $[Na^+]_i$ increase from 39.1 ± 2.2 to 29 ± 6 mM ($n = 4$) (Fig. 3 *c*). Addition of TEA to stimulated cells reduced $[Na^+]_i$ by ~8.7 ± 3.1 mM (Fig. 3 *d*). Fig. 4 shows that a different behavior was observed with secretin-stimulated cells. While TZ (not shown) and TEA (Fig. 4 *a*) had minimal effect, bumetanide significantly inhibited the effect of secretin on $[Na^+]_i$ (Fig. 4 *b*). Because of the slow time course of the secretin effect, the most convincing results

were obtained when bumetanide was added and then removed after stabilization of $[Na^+]_i$. In secretin-stimulated cells, bumetanide reduced $[Na^+]_i$ by about 9.2 ± 1.4 mM ($n = 5$).

Figs. 3 and 4 clearly show that Ca^{2+}-metabolizing agonists and agonists that increase cellular cAMP activate different Na^+ influx mechanisms. These findings were extended by measuring the effect of external K^+ (K_o^+) on the agonist-depen-

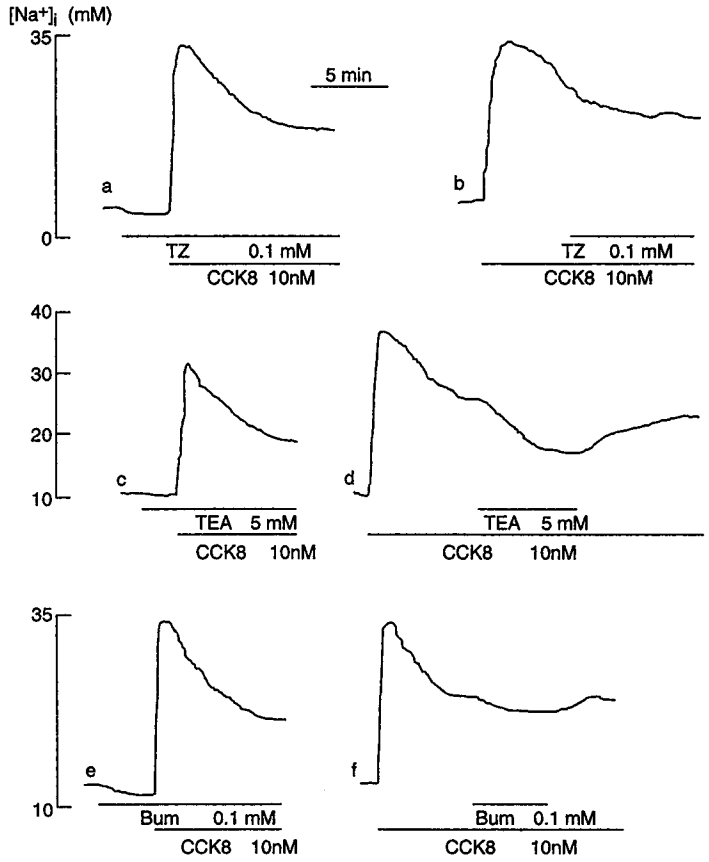

FIGURE 3. Effect of Na^+ influx inhibitors on CCK-dependent increase in $[Na^+]_i$. Acini loaded with SBFI were stimulated with 10 nM CCK8 and exposed to 0.1 mM TZ (*a* and *b*), 5 mM TEA (*c* and *d*), or 0.1 mM bumetanide (*e* and *f*) before or after stimulation with CCK8.

dent $[Na^+]_i$ increase. Changes in K_o^+ are expected to affect several Na^+ transporters such as Na^+ influx through Na^+ permeable channels, Na^+ influx by the NaK2Cl cotransporter and the Na^+/H^+ exchangers and Na^+ efflux by the Na^+ pump. If the two classes of agonists affect different Na^+ transporters, then changes in K_o^+ may differentially affect $[Na^+]_i$ in cells stimulated by each class of agonist. Fig. 5, *a* and *c*, shows that before stimulation the removal of K_o^+ caused a slow increase in $[Na^+]_i$,

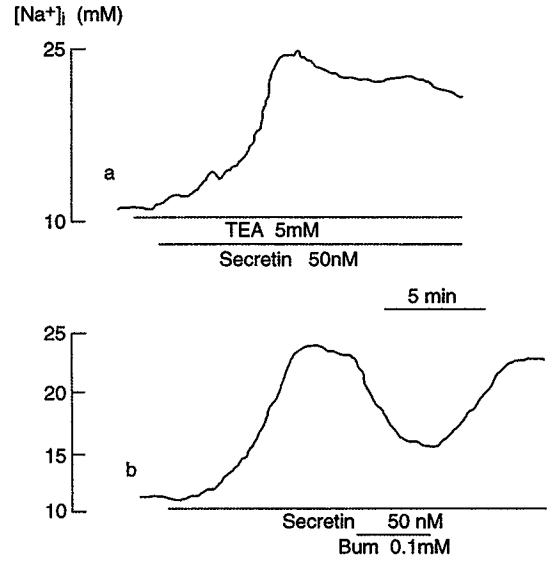

FIGURE 4. Effect of TEA and bumetanide on secretin-dependent increase in $[Na^+]_i$. Acinar cells loaded with SBFI were stimulated with 50 nM secretin (*a* and *b*). In *a*, 5 mM TEA was included in the incubation medium before and during stimulation with secretin. In *b*, where indicated, the perfusion medium contained 0.1 mM bumetanide.

similar to inhibition of the Na^+ pump with ouabain. Stimulation of these cells with carbachol rapidly increased $[Na^+]_i$ to ~68 ± 9 mM (n = 3) (Fig. 5 *a*), which was significantly higher than that measured in the presence of 5 mM K_o^+ (see Fig. 1). Furthermore, $[Na^+]_i$ remained stably elevated until 5 mM K^+ was added to the per-

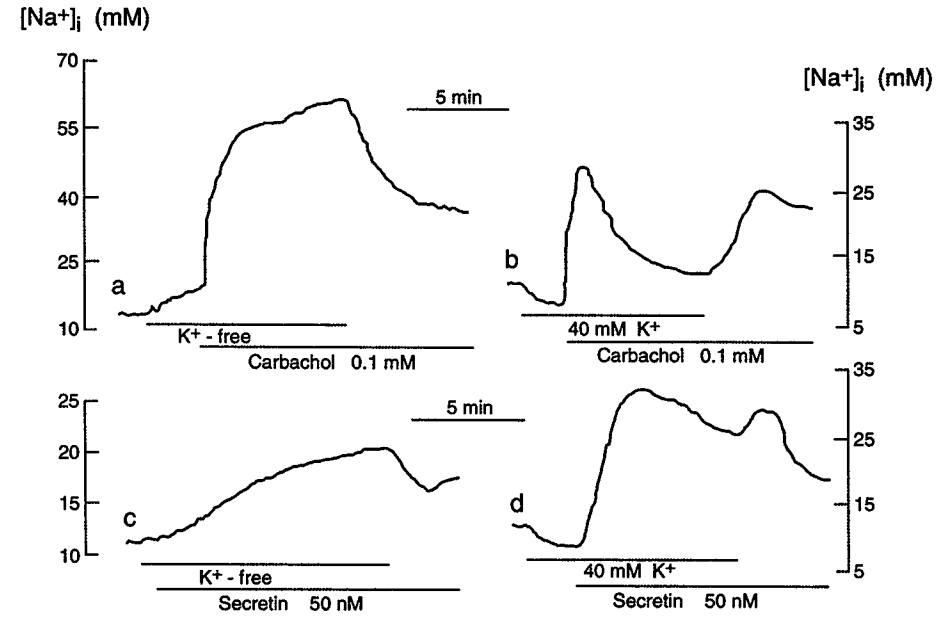

FIGURE 5. Effect of external $[K^+]$ on agonist-stimulated Na^+ influx. Acini loaded with SBFI were perfused with solution D (K^+-free) (*a* and *c*) or solution A in which 35 mM KCl replaced 35 mM NaCl (*b* and *d*) and were then stimulated with 0.1 mM carbachol (*a* and *b*) or 50 nM secretin (*c* and *d*). Where indicated, the cells were then perfused with solution A (5 mM K^+).

fusion medium, which reduced $[Na^+]_i$ to ~33 ± 5 mM (n = 3). The reduction of $[Na^+]$ caused by the readdition of 5 mM K^+ was largely blocked by ouabain (not shown), suggesting the activation of the Na^+-pump. Fig. 5 *c* shows that the opposite was observed with secretin. Removal of K_o^+ significantly reduced the rate and extent of the $[Na^+]_i$ increase. Also with this agonist the addition of K^+ to the medium initially reduced $[Na^+]_i$.

The effect of high K_o^+ an agonist-dependent $[Na^+]_i$ increase is shown in Fig. 5, *b* and *d*. Replacing 35 mM Na^+ with 35 mM K_o^+ reduced resting $[Na^+]_i$ by ~2.85 ± 0.4 mM (n = 14). Although the mechanism responsible for this reduction is not clear at present, it is not attributable to reduction of Na_o^+, because replacing 35 mM Na^+ with 35 mM NMG^+ had no measurable effect on $[Na^+]_i$ (not shown). Stimulation of cells in high K^+ medium with carbachol caused a transient increase in $[Na^+]_i$ from ~8.4 ± 1.1 to 26 ± 3.1 mM, which was then reduced to 14 ± 1.8 mM (Fig. 5 *b*). Reducing K_o^+ back to 5 mM to repolarize the cells increased $[Na^+]_i$ to 22 ± 2.1 mM (n = 3). These findings contrast with the stimulation of the rate and extent of secretin-dependent $[Na^+]_i$ increase by high K_o^+ (Fig. 5 *d*). In the presence of 40 mM K_o^+, secretin increased $[Na^+]_i$ to 31.6 ± 3.5 mM (n = 3). Hence, the experiments with different K_o^+ further indicate that the two agonists stimulate different mechanisms to increase $[Na^+]_i$.

It is evident from Figs. 3–5 that only part of the Na^+ influx is mediated by K_o^+-dependent mechanisms in stimulated cells. The Ca^{2+}-mobilizing agonists were shown to activate the Na^+/H^+ exchanger in acinar cells (Dufresne et al., 1985; Muallem and Loessberg, 1990*b*). Fig. 6 shows the relative contribution of this exchanger to the agonist-dependent $[Na^+]_i$ increase. Incubating the cells with DMA inhibited the rate of CCK-induced $[Na^+]_i$ increase relative to controls measured in different samples of acini from the same cell preparation by ~67% ± 11% (n = 3). Removal of DMA resulted in a further $[Na^+]_i$ increase (Fig. 6, *a* and *b*). Similar results were obtained with carbachol and bombesin (not shown). DMA inhibited the rate of secretin-stimulated Na^+ influx by ~54% ± 4% (n = 3), with an increase in $[Na^+]_i$ after the removal of DMA (Fig. 6, *e* and *f*).

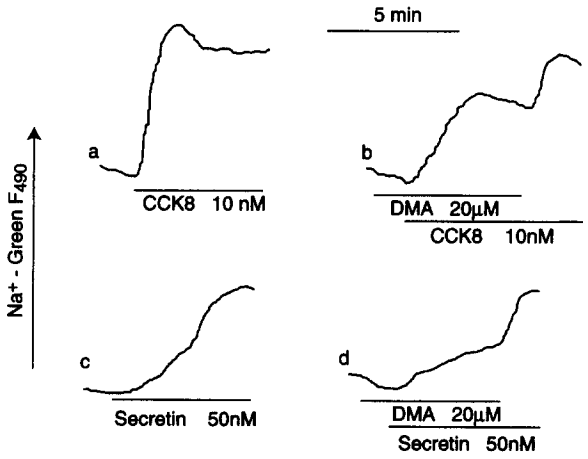

FIGURE 6. Inhibition of agonist-stimulated Na^+ influx by DMA. Acinar cells loaded with Na^+-Green (~83% intracellular dye) were stimulated with 10 nM CCK8 (*a* and *b*) or 50 nM secretin (*c* and *d*). As indicated, 20 μM DMA was included in the perfusion medium.

Relationship Between Transporters in Stimulated Cells

Fig. 7 depicts the effect of the two classes of agonists on ^{86}Rb uptake. Stimulation with carbachol (or CCK8 or bombesin, not shown) had multiple effects on the uptake. Carbachol modestly increased the total uptake by an average of 39% ± 2.7% (n = 8). However, carbachol almost completely prevented the effect of bumetanide on ^{86}Rb uptake and increased the ouabain-sensitive ^{86}Rb uptake to ~44 ± 3.5 nmol/mg protein/4 min, which accounted for ~75% ± 6.8% of the total uptake. In the presence of bumetanide, >96% of the uptake was ouabain sensitive. Secretin

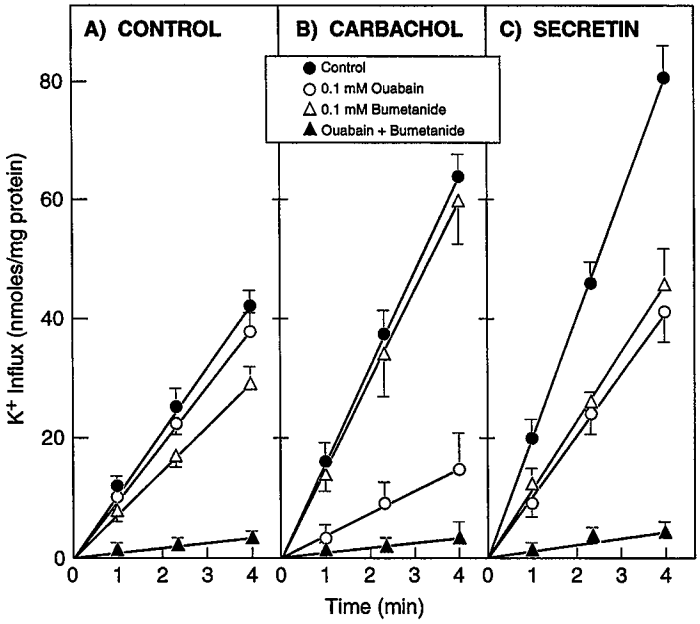

FIGURE 7. Stimulation of ^{86}Rb uptake by agonists. Acini incubated in solution A for 20 min at 37°C were diluted into uptake medium containing ^{86}Rb, the indicated inhibitors (*A*) and 0.1 mM carbachol (*B*), or 50 nM secretin (*C*). At the indicated times, samples were removed to determine ^{86}Rb content of the acini as described in Methods. The points are the mean ± SEM of six to eight experiments performed in duplicate determinations.

had a different effect. Stimulation of the cells with 50 nM secretin increased total ^{86}Rb uptake by 80.5% ± 13% (n = 6) to ~74.4 ± 7.2 nmol/mg protein/4 min. In this case both bumetanide and ouabain inhibited the uptake by about 50% (Fig. 7 *c*).

Since TEA and DMA affected agonist-stimulated $[Na^+]_i$ increase, we tested their effect on ^{86}Rb uptake. Fig. 8 shows the effect of TEA on the uptake in cells stimulated with carbachol or secretin. TEA inhibited total carbachol-stimulated ^{86}Rb uptake by ~8.9% ± 2.7% (n = 4). On the other hand, it reduced the ouabain-sensitive fraction from 39.2% ± 2.5% to 12.1% ± 1.6% in the absence of bumetanide and to 24.2% ± 1.2% in the presence of bumetanide. At the same time, TEA in-

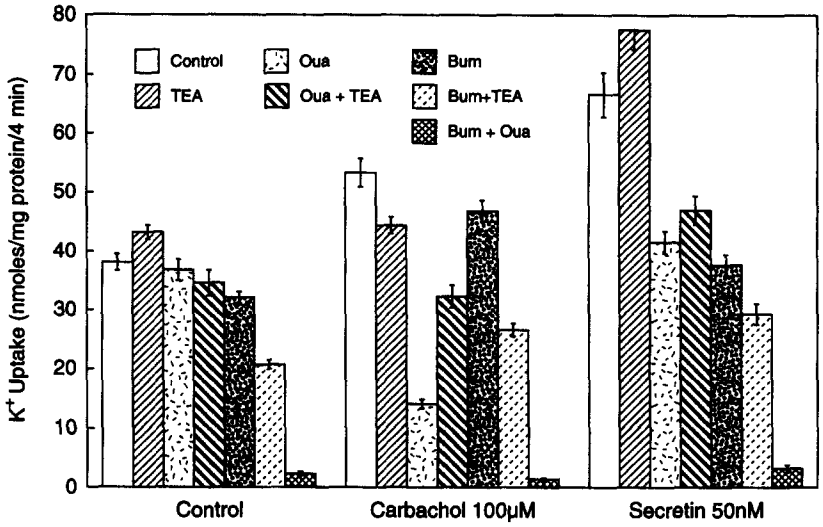

FIGURE 8. Effect of TEA on agonist-stimulated ^{86}Rb uptake. For measurement of ^{86}Rb uptake, acini suspended in solution A and incubated for 20 min at 37°C were diluted 1:1 with solution A containing ^{86}Rb and twice the final desired concentration of TEA (5 mM), ouabain (0.1 mM), and/or bumetanide (0.1 mM) (control cells, *left columns*). In addition, cells were also stimulated with carbachol (100 μM) (*middle columns*) or secretin (50 nM) (*right columns*). After 4 min incubation at 37°C, samples were removed to a stop solution and assayed for ^{86}Rb content. Each column is the mean ± SEM of seven (control), four (carbachol), or three (secretin) experiments performed in duplicate determinations.

creased the bumetanide-sensitive fraction from 6.5% ± 3% to 17.8% ± 1.3% in the absence of ouabain and to 29.9% ± 1.9% in the presence of ouabain. Hence, TEA partially reversed the effect of carbachol on the bumetanide- and ouabain-sensitive fractions of ^{86}Rb uptake. This was even more evident when the overlapped fractions were considered. In contrast, TEA had a marginal effect on ^{86}Rb uptake in the presence of secretin. Thus, TEA slightly increased the uptake in the absence of blockers, had no effect on the ouabain-sensitive fraction, and somewhat augmented the bumetanide-sensitive uptake.

Fig. 9 shows that DMA had profound effect on ^{86}Rb uptake under all conditions. Whereas DMA increased ^{86}Rb uptake into control cells, it inhibited the uptake into carbachol-stimulated cells by 23% ± 3.2% (n = 4) and the uptake into secretin-stimulated cells by 16% ± 1.8% (n = 4). In the presence of DMA, ouabain and bumetanide alone almost completely inhibited the uptake, whether the cells were stimulated with carbachol or secretin. Hence, a functioning Na^+/H^+ exchanger is essential for K^+ uptake into stimulated cells.

Role of Second Messengers

To examine the role of cAMP in the response to secretin, we measured the effect of forskolin on $[Na^+]_i$ and ^{86}Rb uptake. Fig. 10 shows that for the most part forskolin acted like secretin. Forskolin slowly increased $[Na^+]_i$ (Fig. 10 *a*). This effect was in-

hibited in part by bumetanide (Fig. 10 *b*) and in part by DMA (Fig. 10 *c*), and the effect of DMA appeared more prominent than that of bumetanide. Forskolin increased the rate of ^{86}Rb uptake to the same extent found with secretin (69.2 ± 4.3 and 75.4 ± 4.6 nmol/mg protein/4 min, respectively), and the uptake was inhibited 45–50% by ouabain or bumetanide. In the presence of DMA, ouabain or bumetanide almost completely inhibited ^{86}Rb uptake in forskolin-treated cells (Fig. 10, *left panel*). Hence, it is likely that the effect of secretin on $[Na^+]_i$, ^{86}Rb uptake, and the other transporters was mediated by an increase in cellular cAMP.

It is commonly accepted that the effect of Ca^{2+}-mobilizing agonists on cellular functions is the result of activation of Ca^{2+}-dependent mechanisms and of protein kinase C (PKC) (Berridge, 1993). The experimental tools used to evaluate the contribution of each pathway are the inhibitor of internal Ca^{2+} pumps, thapsigargin (Tg), which increases $[Ca^{2+}]_i$, and the tumor promoting phorbol ester, TPA, which activates PKC. Fig. 11 shows the effect of Tg on $[Ca^{2+}]_i$ and $[Na^+]_i$ under similar conditions. Tg increased $[Ca^{2+}]_i$ in these cells to about 340 nM within 60–70 s, after which $[Ca^{2+}]_i$ was slowly reduced but remained elevated until the cells were stimulated with CCK8 (Fig. 11 *a*). In such cells, CCK8 actually reduced $[Ca^{2+}]_i$ because of activation of the plasma membrane Ca^{2+} pump (see Zhang, Zhao, Loessberg, and Muallem, 1992). Surprisingly, Tg caused only a slow and small increase in $[Na^+]_i$ (Fig. 11 *b*). After 2, 6 and 12 min of incubation, Tg increased $[Na^+]_i$ from 11.6 ± 1.7 to 14.2 ± 1.7, 16.6 ± 2.4, and 22.3 ± 3.1 mM (n = 4), respectively. Stimulation of these cells with CCK8 had no further effect on $[Na^+]_i$. On the other hand, when the cells were treated with Tg for 2 min and then stimulated with

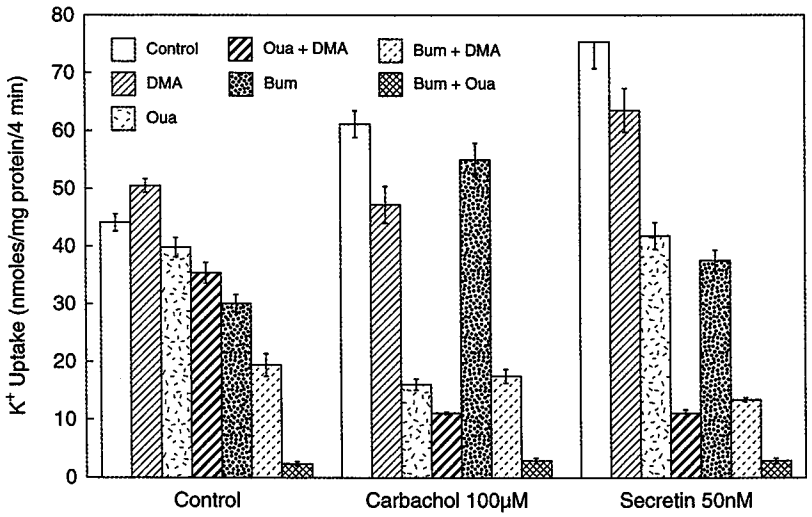

FIGURE 9. Effect of DMA on agonist-stimulated ^{86}Rb uptake. ^{86}Rb uptake was measured as described in the legend of Fig. 8, except that where indicated, 20 μM DMA was used to inhibit the Na^+/H^+ exchanger. Each column is the mean ± SEM of eight (control) or four (carbachol or secretin) experiments performed in duplicate determinations.

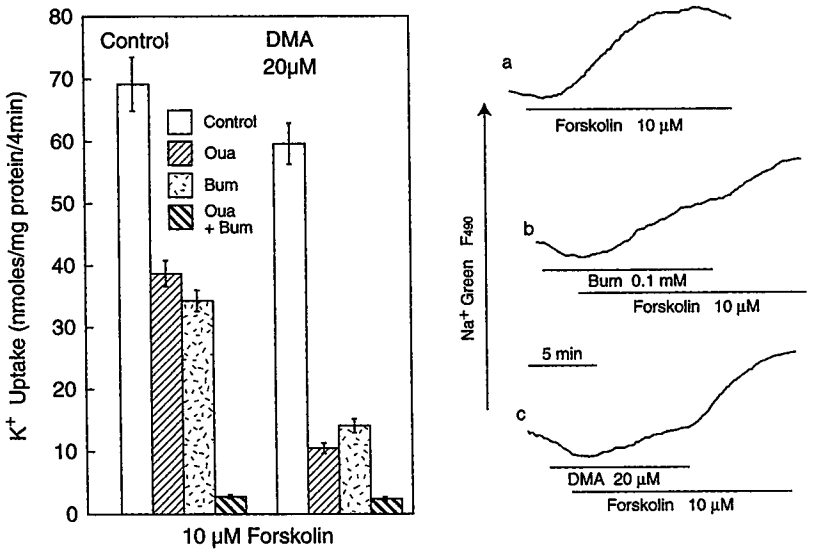

FIGURE 10. Stimulation of Na^+ influx and ^{86}Rb uptake by forskolin. For measurements of Na^+ influx, acini were loaded with Na^+-Green and stimulated with 10 μM forskolin. The perfusion medium (solution A) also contained 0.1 mM bumetanide or 20 μM DMA as indicated. ^{86}Rb uptake was measured as described in the legend to Fig. 8, except that the cells were stimulated with 10 μM forskolin. The columns on the left represent control cells stimulated with forskolin and the columns on the right represent experiments performed in the presence of 20 μM DMA. Each column is the mean ± SEM of three experiments performed in duplicate determinations.

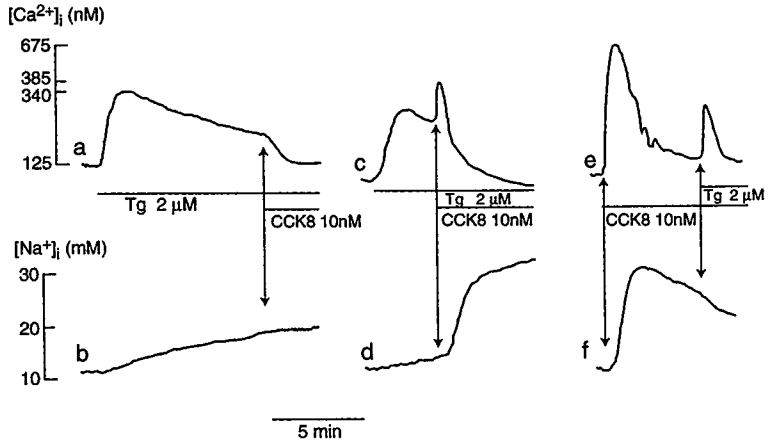

FIGURE 11. Relationship between Tg-mediated increase in $[Ca^{2+}]_i$ and $[Na^+]_i$. Acini loaded with Fura 2 (*a*, *c*, and *e*) or SBFI (*b*, *d*, and *f*) were treated with 2 μM Tg (*a* and *b*), Tg and then 10 nM CCK8 (*c* and *d*), or CCK8 and then Tg (*e* and *f*) as indicated. $[Ca^{2+}]_i$ and $[Na^+]_i$ were measured with different acini from the same preparation and stimulation time was the same to facilitated comparison between $[Ca^{2+}]_i$ and $[Na^+]_i$ changes.

CCK8, $[Ca^{2+}]_i$ increased to ~385 nM (Fig. 11 *C*), whereas $[Na^+]_i$ increased to levels measured in control cells (compare Fig. 11, *D* and *F*). The increase in $[Na^+]_i$ always lagged behind the increase in $[Ca^{2+}]_i$ (Fig. 11, *c–f*).

The effect of Tg and CCK8 on ^{86}Rb uptake is shown in Fig. 12. Pretreatment of the cells with Tg for 2 or 6 min had minimal effect on maximal ^{86}Rb uptake or the relative effects of ouabain and bumetanide on the uptake. Treatment of the cells with Tg for 2 min, which partially depleted stored Ca^{2+} (Fig. 11 *c*) without preventing the effect of CCK8 on $[Na^+]_i$ (Fig. 11 *d*), also did not prevent the effect of CCK8 on ^{86}Rb uptake. Thus, in cells treated with Tg for 2 min, CCK8 increased the total and the fraction of ouabain-sensitive ^{86}Rb uptake and inhibited the bumet-

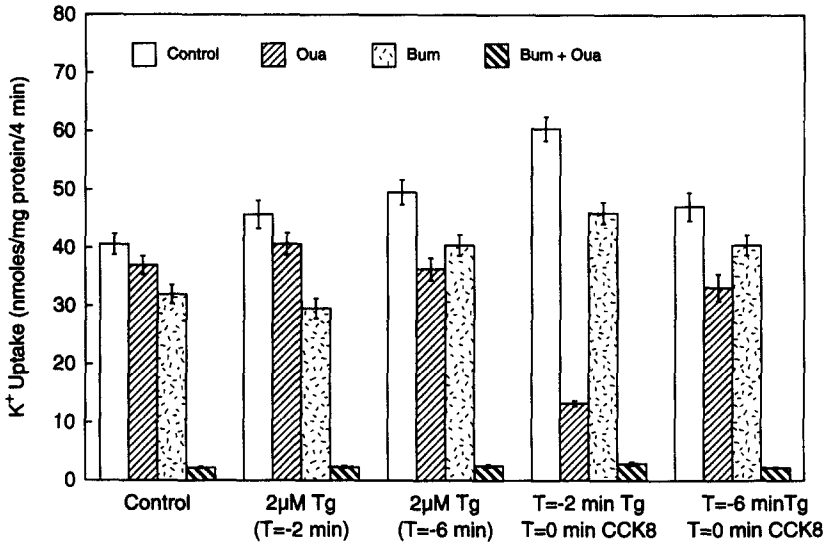

FIGURE 12. Effect of pretreatment with Tg on ^{86}Rb uptake. Acini in solution A were incubated for 20 min at 37°C. During the last 2 or 6 min of the incubation, samples of acini were treated with 2 μM Tg as indicated. ^{86}Rb uptake was initiated by a 1:1 dilution of control or Tg-treated cells into incubation medium that also contained Tg or Tg and 10 nM CCK8 as indicated. After 4 min incubation at 37°C, duplicate samples were removed to determine ^{86}Rb content of cells. Each column is the mean ± SEM of five separate experiments.

anide-sensitive uptake (compare the second and fourth groups in Fig. 12). Treating the cells with Tg for 6 min, which prevented the effect of CCK8 on $[Ca^{2+}]_i$ (Fig. 11 *a*) and $[Na^+]_i$ (Fig. 11 *b*) also inhibited the effects of CCK8 on ^{86}Rb uptake (compare the third and fifth groups in Fig. 12). Hence although an increase in $[Ca^{2+}]_i$ was not sufficient to activate the mechanisms of Na^+ influx, it was required for the agonist to activate these mechanisms.

The role of PKC in mediating the effect of the agonist was evaluated by measuring the effect of TPA on $[Na^+]_i$ and ^{86}Rb uptake. Fig. 13 shows that pretreatment of the cells with 1 μM TPA stimulated ^{86}Rb uptake in a time-dependent manner. After 6 min stimulation with TPA, ^{86}Rb uptake increased from 38.3 ± 1.4 to 47.7 ± 2.1

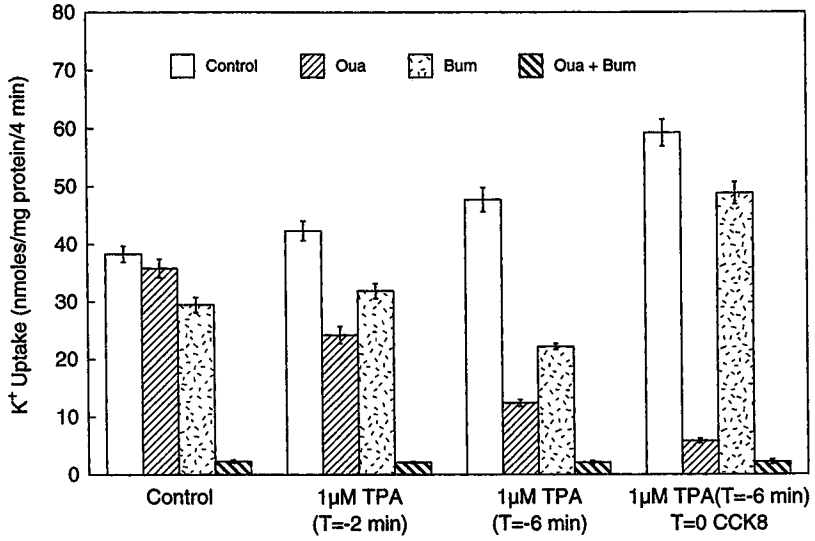

FIGURE 13. Effect of pretreatment with TPA on ^{86}Rb uptake. Experimental protocols were similar to those in Fig. 12 except that the cells were treated with 1 μM TPA for 2 or 6 min before measurements of ^{86}Rb uptake in the presence or absence of TPA or CCK8 as indicated. Each point is the mean ± SEM of three experiments.

nmol/mg protein/4 min. Importantly, TPA treatment made the ^{86}Rb uptake sensitive to ouabain and bumetanide. Hence, functioning Na^+ pump and NaK2Cl cotransport were required for ^{86}Rb uptake in TPA-treated cells. The results in Fig. 13, last group, show that stimulation of PKC with TPA did not prevent the overall

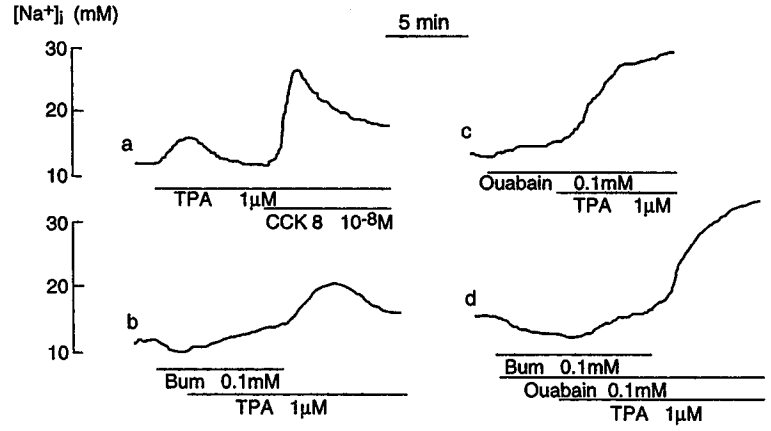

FIGURE 14. Effect of TPA on $[Na^+]_i$. Pancreatic acinar cells loaded with SBFI were stimulated with 1 μM TPA and then 10 nM CCK8 (*a*). In *b* cells were treated with 0.1 mM bumetanide before stimulation with TPA. In *c* and *d* the cells were incubated with 0.1 mM ouabain (*c* and *d*) and 0.1 mM bumetanide (*d*) before stimulation with TPA. In *d*, removal of bumetanide caused a large increase in $[Na^+]_i$.

effect of CCK8 on ^{86}Rb uptake. Hence, stimulation of bumetanide-sensitive uptake by TPA did not prevent the inhibition of this activity by agonist stimulation.

Fig. 14 shows that stimulation of the cells with 1 μM TPA caused a transient increase in $[Na^+]_i$. TPA increased $[Na^+]_i$ by ~4.8 ± 0.7 mM (n = 5) within 2 min of stimulation, but after ~5 min $[Na^+]_i$ returned to near resting levels. Stimulation of these cells with CCK8 rapidly increased $[Na^+]_i$ to ~29 ± 1.9 (n = 3) (Fig. 14 *a*). The results in Fig. 13 suggest that in TPA-treated cells, part of the ^{86}Rb uptake was mediated by the NaK2Cl cotransporter, which also provided a significant amount of Na^+ for the Na^+ pump. Accordingly, Fig. 14 *b* shows that bumetanide largely inhibited the effect of TPA on $[Na^+]_i$. Fig. 14 *c* shows that inhibition of the Na^+ pump with ouabain strongly augmented the effect of TPA which increased $[Na^+]_i$ to 26.6 ± 2.7 mM (n = 3). Again, this effect of TPA on $[Na^+]_i$ was strongly inhibited by bumetanide (Fig. 14 *d*). The combined results in Figs. 13 and 14 indicate that stimulation of PKC cannot explain the effect of the Ca^{2+}-mobilizing agonists on Na^+ influx.

Another form of activation or modulation of the transporters mediating Na^+ influx is by changes in cell volume. It is possible that stimulation of pancreatic acinar cells with Ca^{2+}-mobilizing agonists caused cell shrinkage and subsequent Na_o^+-dependent cell swelling, similar to that reported in salivary acinar cells (Foskett and Melvin, 1989). We therefore tested the effect of cell volume changes on Na^+ and

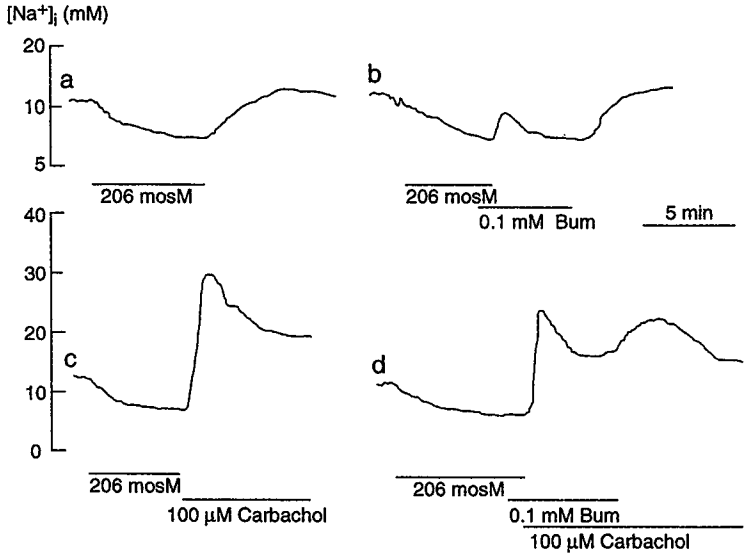

FIGURE 15. Cell volume-dependent changes in $[Na^+]_i$. Acinar cells loaded with SBFI were perfused with a solution of 206 mosM, which had the same composition as solution A except for the reduction of NaCl from 145 to 93 mM (*a–d*). In *a*, after incubation in hypotonic medium, osmolarity was increased back to 310 mosM by perfusion with solution A. In *b*, 0.1 mM bumetanide was included in the solution used for cell shrinkage. Where indicated, bumetanide was removed from the incubation medium while maintaining osmolarity at 310 mosM. In *c*, the cells were stimulated with 0.1 mM carbachol during cell shrinkage. In *d*, 0.1 mM bumetanide was included in solution A.

K^+ fluxes in control and agonist-stimulated cells. An important aspect of these experiments was to determine whether the agonist can inhibit the NaK2Cl cotransport after its activation by cell shrinkage. Fig. 15 shows that swelling the cells by reducing medium osmolarity from 310 to 206 mosM caused a biphasic reduction in $[Na^+]_i$. The rapid, initial reduction from 11.3 ± 0.9 to 7.5 ± 0.7 mM was completed within 60–75 s. In the subsequent 5 min of incubation in hypotonic medium, $[Na^+]_i$ was slowly reduced to ~6.0 ± 0.75 mM (n = 15) (Fig. 15, *a–d*). Shrinking the cells by restoring the osmolarity to 310 mosM gradually increased $[Na^+]_i$ back toward resting levels (Fig. 15 *a*). This effect was partially blocked by bumetanide (Fig. 15 *b*) in that cell shrinkage in the presence of bumetanide caused a transient increase in $[Na^+]_i$. At present we do not know what mechanism is responsible for this unusual $[Na^+]_i$ increase. However, removal of bumetanide from the medium of these cells restored normal $[Na^+]_i$ (Fig. 15 *b*). Swelling the cells by exposure to low osmolarity did not prevent the agonist-induced $[Na^+]_i$ increase on cell shrinkage (Fig. 15 *c*). Furthermore, bumetanide did not prevent the $[Na^+]_i$ increase under these conditions (Fig. 15 *d*). The properties of ^{86}Rb uptake under the conditions of Fig. 15 are depicted in Fig. 16. As was reported before for many cell types (Hoffman and Simonsen, 1989; Grinstein and Foskett, 1990), subjecting pancreatic acini to a swell–shrink cycle stimulated ^{86}Rb uptake from ~41.8 ± 3.3 to 83.4 ± 3.8 nmol/mg protein/4 min (n = 4). This was largely due to the stimulation of the NaK2Cl cotransporter, since bumetanide inhibited the uptake by 54 ± 2.6% and 76 ± 3.2% in the absence and presence of ouabain, respectively. Stimulation of the cells with carbachol (or CCK8) further increased the uptake to 101.3 ± 4.6 nmol/mg protein/4 min. Carbachol stimulation also increased the fraction of ouabain-sensitive ^{86}Rb uptake and inhibited the fraction of bumetanide-sensitive uptake in these cells. Hence, stimulation of the NaK2Cl cotransporter by cell shrinkage did not prevent its inhibition by agonist stimulation.

DISCUSSION

Characterization of Na^+, K^+, Cl^- (Maruyama and Petersen, 1982; O'Doherty and Stark, 1983; Zhao and Muallem, 1995), HCO_3^- and H^+ (Muallem and Loessberg, 1990*a*) fluxes in pancreatic acinar cells showed that multiple transport pathways determine the concentration of these ions in the cytosol. The various pathways are regulated by, and coupled through, the cytosolic concentration of the transported ions. Thus, it is well documented that the Na^+/H^+ exchanger is regulated by $[H^+]_i$ (Grinstein and Foskett, 1990) and $[Na^+]_i$ (Green, Yamaguchi, Kleeman, and Muallem, 1988). The Cl^-/HCO_3^- exchanger is regulated by HCO_3^- and pHi (Green et al., 1990), and the NaK2Cl cotransporter is sensitive to $[Na^+]_i$ (Whisenant, Khademazad, and Muallem, 1991). Furthermore, the transport pathways complement and compensate each other to regulate the concentration of the transported ions, in particular $[Na^+]_i$ and $[K^+]_i$ (Zhao and Muallem, 1995). In the present studies we were able to demonstrate agonist-specific and selective stimulation of ion transporters and show the importance of $[Na^+]_i$ in the regulation of fluid and electrolyte secretion by pancreatic acini.

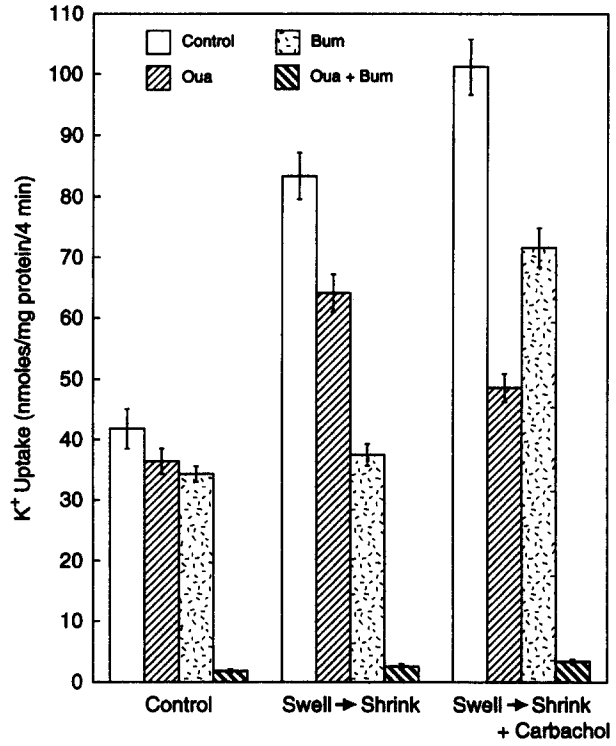

FIGURE 16. Stimulation of ^{86}Rb uptake by cell shrinkage. Acinar cells were incubated with solution A for 20 min at 37°C. At 14 min, a sample of the cells was mixed with a solution containing all additives of solution A except NaCl to obtain a final NaCl concentration of 93 mM. At the end of the 20 min incubation at 37°C, cells incubated in solution A (isotonic) were diluted (1:1) into solution A (*left columns*, control) and cells incubated in hypotonic media were diluted into solution A containing 197 mM NaCl to obtain a final osmolarity of 310 mosM (*middle columns*, swell/shrink), and also containing 0.1 mM carbachol (*right columns*). The incubation solutions to which the cells were diluted also contained ^{86}Rb and as indicated 0.1 mM ouabain, 0.1 mM bumetanide, or both. After 2 and 4 min incubation at 37°C, duplicate samples were removed to determine ^{86}Rb content of cells. Since uptake was linear for 4 min at 37°C, all samples were averages and calculated as uptake/4 min incubation. Each column is the mean ± SEM of four similar experiments performed at quadruplicate determinations.

Specificity of the $[Na^+]_i$ Increase

Multiple transporters were activated to cause the agonist-induced increase in $[Na^+]_i$. Ca^{2+}-mobilizing agonists activated the Na^+/H^+ exchanger and the nonselective cation channels. A significant part of the $[Na^+]_i$ increase was inhibited by DAM (Fig. 6). This agrees with previous studies demonstrating the activation of the Na^+/H^+ exchanger and H^+ efflux by Ca^{2+}-mobilizing agonists (Dufresne et al., 1985; Muallem and Loessberg, 1990*b*). Na^+ influx by the nonselective channel is concluded from the increase in Na^+ influx in the absence of K_o^+, a decrease in the

influx at high K_o^+, the partial inhibition of the influx by TEA, and from the effect of TEA on ^{86}Rb uptake. Previous studies showed that Ca^{2+}-mobilizing agonists activate a nonselective cation channel in rat and mouse pancreatic acini (Marayama and Petersen, 1982; Thorn and Petersen, 1992). Similar channels and their activation by Ca^{2+}-mobilizing agonists have been reported in many secretory cell types (Petersen and Findlay, 1987; Petersen, 1992). However, the role of these channels in secretion remained unknown. Here we show that a major role of the channels is to provide a pathway for Na^+ influx during agonist stimulation, although the contribution of the channels is secondary to that of the Na^+/H^+ exchanger.

Unlike previous reports in mouse pancreatic acini (Petersen and Singh, 1985), there was no evidence for stimulation of the rat pancreatic acinar NaK2Cl cotransporter by Ca^{2+}-mobilizing agonists. Measurements of ^{86}Rb influx showed that the cotransporter is actually inhibited by this class of agonists. This agrees with studies

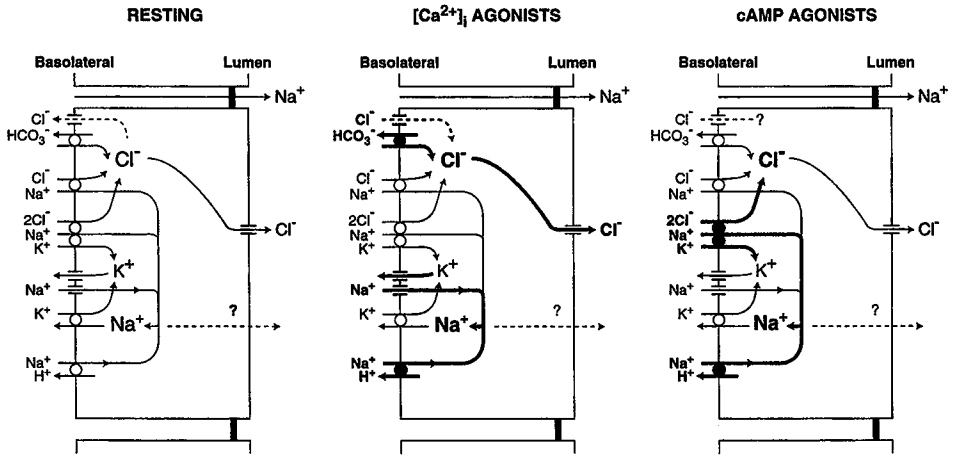

FIGURE 17. A model of ion transport mechanisms in rat pancreatic acinar cells. In the middle and left columns, the transporters shown to be stimulated by Ca^{2+}-mobilizing agonist and agonists that increase cellular cAMP, respectively, are highlighted.

showing no effect of furosemide on fluid secretion by the perfused rat pancreas (Ishikawa and Kanno, 1991). Species differences with respect to mechanism of pancreatic fluid and electrolyte secretion are well documented (Case and Argent, 1986; Petersen, 1992) and may explain the differences between the studies in the rat and mouse acinar cells.

The consequence of stimulation of Na^+ influx by the Ca^{2+}-mobilizing agonists was an increased Na^+ pump activity and inhibition of the NaK2Cl cotransporter (see Fig. 17, *middle*). Our previous studies showed that the NaK2Cl cotransporter is highly sensitive to $[Na^+]_i$ (Whisenant et al., 1993). It is therefore likely that the increase in $[Na^+]_i$ inhibited the cotransporter and stimulated the Na^+ pump. This interpretation is supported by the experiments with TEA (Fig. 8) and DMA (Fig. 9). TEA, which partially inhibited Na^+ influx, reduced the fraction of ouabain-sensitive

^{86}Rb influx and increased the fraction of bumetanide-sensitive ^{86}Rb influx. Inhibition of Na^+ influx by the Na^+/H^+ exchanger with DMA has a small effect on total ^{86}Rb uptake in stimulated cells. However, in the presence of DMA, most Na^+ influx was mediated by the cotransporter since bumetanide largely inhibited ^{86}Rb influx. This is probably because Na^+ influx through the TEA-sensitive channel was not sufficient to support the Na^+ pump. In the presence of DMA, ouabain alone effectively inhibited ^{86}Rb uptake in stimulated but not resting cells. Most likely this is because inhibition of the Na^+ pump in stimulated cells resulted in large $[Na^+]_i$ increases, which inhibited the cotransporter.

Stimulation of the cells with secretin also increased Na^+ influx. However, with this agonist the influx was mediated by the Na^+/H^+ exchanger and the NaK2Cl cotransporter. Accordingly, DMA or bumetanide reduced the effect of secretin on $[Na^+]_i$ increase, whereas TEA (Figs. 4 and 8) or TZ (not shown) had minimal effect on $[Na^+]_i$ or ^{86}Rb fluxes stimulated by secretin. Ouabain or bumetanide inhibited ~50% of ^{86}Rb influx in secretin-stimulated cells. This suggests that secretin uncouples K^+ uptake by the two transporters. This is probably not the case since DMA alone only slightly inhibited the influx, whereas in the presence of DMA both transporters must be functional for K^+ uptake to occur. Thus, in the presence of DMA, transport by the Na^+ pump and the NaK2Cl cotransporter appears tightly coupled.

Role of Second Messengers

Secretin was reported to increase cAMP levels (Gardner and Jensen, 1986) and $[Ca^{2+}]_i$ (Trimble, Bruzzone, Biden, and Farese, 1986) in pancreatic acinar cells. However, the present studies show that the overall effect of secretin on the transporters can be induced by an increase in cAMP with forskolin (Fig. 10). Measurements of $[Ca^{2+}]_i$ in our laboratory showed that in acinar cells, secretin increased $[Ca^{2+}]_i$ to ~35% that found with carbachol, whether $[Ca^{2+}]_i$ was measured in cell suspension or with single acinar cells (results not shown). It is likely that the degree of activation of the Ca^{2+} signaling pathway by secretin was not sufficient to activate the same transporters activated by the Ca^{2+}-mobilizing agonists.

We were unable to determine with certainty the mechanism by which Ca^{2+}-mobilizing agonists activate the different Na^+ transport pathways. As reported in previous studies (Hootman et al., 1987), stimulation of PKC with TPA increased the rate of ^{86}Rb uptake. However, this was not attributable to direct stimulation of the Na^+ pump, as suggested before (Hootman et al., 1987), but rather to stimulation of the NaK2Cl cotransporter, and consequently K^+ influx by the Na^+ pump. Furthermore, the agonist, through an increase in $[Na^+]_i$, inhibited bumetanide-sensitive ^{86}Rb influx in TPA-treated cells. Even more surprising was the lack of effect of Tg. Tg releases Ca^{2+} from the agonist-sensitive pool to increase $[Ca^{2+}]_i$ in the same sites or compartments as the agonists (Zhang et al., 1992). In salivary acinar cells, increasing $[Ca^{2+}]_i$ with Tg was equivalent to agonist stimulation in activating several Na^+ influx pathways (Robertson and Foskett, 1994). Therefore, we expected Tg to act like agonists, at least in stimulating the TEA-sensitive, Ca^{2+}-activated pathway. However, in pancreatic acinar cells, Tg caused a slow and small increase in $[Na^+]_i$. Treatment of the cells with Tg and TPA also failed to cause agonist-like stimulation of Na^+ influx or ^{86}Rb uptake (not shown).

Despite the lack of effect of Tg, $[Ca^{2+}]_i$ increase by the agonist appears to be required for stimulation of $[Na^+]_i$ influx and ^{86}Rb uptake. Thus, depletion of internal stores Ca^{2+} by a prolonged treatment with Tg prevented the effect of agonist on Na^+ influx. On the other hand, increasing $[Ca^{2+}]_i$ by agonists to levels similar to those induced by Tg was sufficient to activate Na^+ and ^{86}Rb influx (Fig. 11, *c* and *d*, and Fig. 12). It is therefore clear that in pancreatic acinar cells the Ca^{2+}-mobilizing agonists increase $[Ca^{2+}]_i$ and modify the activity of another biochemical pathway to stimulate Na^+ influx and fluid secretion. This pathway seems to be different from a PKC, or a cell volume-sensitive pathway. In salivary acinar cells activation of Na^+ influx is coupled to changes in cell volume (Wong and Foskett, 1991). We therefore tested whether activation of Na^+ influx by similar changes in cell volume will resemble activation by Ca^{2+}-mobilizing agonists. This was not the case in pancreatic acinar cells. In fact, despite the stimulation of the NaK2Cl cotransport and the Na^+ pump by cell shrinkage, the agonists increased $[Na^+]_i$ to further stimulate the pump and inhibit the cotransporter. The biochemical/signaling pathway mediating the activation of Na^+ and ^{86}Rb influx by Ca^{2+}-mobilizing agonists in pancreatic acinar cells remains to be identified.

Models of Fluid and Electrolyte Secretion

The present studies provide sufficient information to propose a model for electrolyte and fluid secretion by rat pancreatic acinar cells when stimulated with Ca^{2+}-mobilizing agonists or agonists that increase cAMP, such as secretin or VIP. The models are summarized in Fig. 17. Na^+ and Cl^- influx at the BLM are the key for regulation of the secretion. In the presence of HCO_3^- most Cl^- influx is mediated by the Cl^-/HCO_3^- exchanger, which dominates Cl^- fluxes at the BLM and is stimulated by Ca^{2+}-mobilizing agonists (Muallem and Loessberg, 1990*b*). The Na^+/H^+ exchanger is also activated to increase Na^+ influx to fuel the Na^+ pump and remove the H^+ generated in the cytosol by Cl^-/HCO_3^- exchange. Such a mechanism can explain why, in the presence of HCO_3^-, CCK-stimulated fluid secretion by acinar cells is sensitive to low concentrations of SITS and DIDS (Seow, Lingard, and Young, 1986). In the absence of HCO_3^-, when the Cl^-/HCO_3^- exchanger is not active (Muallem and Loessberg, 1990*a*), acinar cells secrete well when stimulated with Ca^{2+}-mobilizing agonists (Petersen and Ueda, 1977; Evans, Pirani, Cook, and Young, 1986; Seow et al., 1986). In this case Cl^- influx in the BLM must be mediated by an alternative pathway. The present studies show that this pathway is not likely to be the NaCl or the NaK2Cl cotransporters. Previous studies, which correlated between changes in $[Ca^{2+}]_i$ and activation of Cl^- current in acinar cells, suggested the sequential activation of Cl^- channels located in the AM and the BLM (Kasai and Augustine, 1990). It is therefore possible that, in the absence of HCO_3^-, a Cl^- channel in the BLM together with the nonselective cation channel provide the route for Cl^- entry across the BLM. Considering the high $[Cl^-]_i$ in acinar cells, another advantage of activation of the nonselective cation channel by the agonist is to depolarize the BLM and thus permit the Cl^- influx.

The effect of cAMP on the Cl^-/HCO_3^- exchanger is not known. Hence, at present it is not clear what role the Cl^-/HCO_3^- exchanger plays during stimulation

of fluid and electrolyte secretion by cAMP modulating agonists. However, with these agonists, at least part of the Cl^- influx in the presence of HCO_3^- and probably most of the influx in the absence of HCO_3^- is mediated by the NaK2Cl cotransporter, which also provides significant fraction of the Na^+ required to fuel the Na^+ pump.

The models in Fig. 17 do not account for the portion (up to 30%) of transcellular Na^+ secretion, since the mechanism responsible for Na^+ efflux at the AM is not known. However, it is of note that with all agonists, $[Na^+]_i$ remained elevated for the duration of cell stimulation, which can facilitate Na^+ efflux across the AM. Further studies obviously are required to identify the Na^+ efflux mechanism at the AM and test the validity of the models in Fig. 17.

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REFERENCES

Bastie, M.-J., M. Delvaux, M. Dufresne, J. S. Saunier-Blache, N. Vaysse, and A. Ribet. 1988. Distinct activation of Na^+/H^+ exchange by gastrin and CCK peptide in acini from guinea pig. *American Journal of Physiology.* 254:G25–G32.

Berridge, M. J. 1993. Inositol trisphosphate and calcium signaling. *Nature.* 361:315–325.

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, J. D. Gardner, F. P. Brooks, E. Lebenthal, E. P. DiMango, and G. A. Sheele, editors. Raven Press, New York. 213–242.

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., and J. E. Melvin. 1989. Activation of salivary secretion. Coupling of cell volume and $[Ca^{2+}]_i$ in single cells. *Science.* 244:1582–1585.

Green, J., D. T. Yamaguchi, C. R. Kleeman, and S. Muallem. 1988. Cytosolic pH regulation in osteoblasts: interaction of Na^+ and H^+ with the extracellular and intracellular faces of the Na^+ and H^+ exchanger. *Journal of General Physiology.* 92:239–261.

Green, J., D. T. Yamaguchi, C. R. Kleeman, and S. Muallem. 1990. Cytosolic pH regulation in osteoblasts: regulation of anion exchange by intracellular pH and Ca^{2+} ions. *Journal of General Physiology.* 95:121–145.

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

Hoffman, E. K., and L. O. Simonsen. 1989. Membrane mechanisms in volume and pH regulation in vertebrate cells. *Physiological Review.* 69:315–382.

Hootman, S. R., M. E. Brown, and J. A. Williams. 1987. Phorbol esters and A23187 regulate Na^+/K^+ pump activity in pancreatic acinar cells. *American Journal of Physiology.* 252:G499– G505.

Hootman, S. R., S. A. Ernest, and J. A. Williams. 1983. Secretagogue regulation of Na^+/K^+ pump ac-

tivity in pancreatic acinar cells. *American Journal of Physiology.* 245:G339–G346.

Hootman, S. R., D. L. Ochs, and J. A. Williams. 1985. Intracellular mediators of Na^+/K^+ pump activity in guinea pig pancreatic acinar cells. *American Journal of Physiology.* 249:G470–G478.

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.

Kasai, H., and G. J. Augustine. 1990. Cytosolic Ca^{2+} gradients triggering unidirectional fluid secretion from exocrine pancreas. *Nature.* 348:735–738.

Maruyama, Y., and O. H. Petersen. 1982. Cholecystokinin activation of single channel currents is mediated by internal mesenger in pancreatic acinar cells. *Nature.* 300:61–63.

Muallem, S., and P. A. Loessberg. 1990*a*. Intracellular pH-regulatory mechanism in pancreatic acinar cells: I. Characterization of H^+ and HCO_3^- transporters. *Journal of Biological Chemistry.* 265:12806–12812.

Muallem, S., and P. A. Loessberg. 1990*b*. Intracellular pH-regulatory mechanisms in pancreatic acinar cells: II. Regulation of H^+ and HCO_3^- transporters by Ca^{2+}-mobilizing agonists. *Journal of Biological Chemistry.* 265:12813–12819.

O'Doherty, J., and R. J. Stark. 1983. A transcellular route for Na-coupled Cl transport in secreting pancreatic acinar cells. *American Journal 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 (London).* 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(3):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.

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.

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.

Trimble, E. R., R. Bruzzone, T. J. Biden, and R. V. Farese. 1986. Secretin induces rapid increases in inositol trisphosphate, cytosolic Ca^{2+} and diacylglycerol as well as cyclic AMP in rat pancreatic acini. *Biochemical Journal.* 239:257–261.

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.

Wong, M. M. Y., and K. J. Foskett. 1991. Oscillations of cytosolic sodium during calcium oscillations in exocrine acinar cells. *Science.* 254:1014–1016.

Zhang, B.-X., H. Zhao, P. A. Loessberg, and S. Muallem. 1992. Activation of the plasma membrane Ca^{2+}-pump during agonist stimulation of pancreatic acini. *Journal of Biological Chemistry.* 267:15419–15425.

Zhao, H., and S. Muallem. 1995. Na^+, K^+, and Cl^- transport in resting pancreatic acinar cells. *Journal of General Physiology.* 106:1225–1242.