Membrane Localization of H\(^+\) and HCO\(_3^-\) Transporters in the Rat Pancreatic Duct

HONG ZHAO,* ROBERT A. STAR, and SHMUEL MUALLEM*

From the *Department of Physiology and †Department of Internal Medicine, The University of Texas Southwestern Medical Center, Dallas, Texas 75235-9040

ABSTRACT The pancreatic duct secretes alkaline fluid that is rich in HCO\(_3^-\) and poor in Cl\(^-\). The molecular mechanisms that mediate ductal secretion and are responsible for the axial gradients of Cl\(^-\) and HCO\(_3^-\) along the ductal tree are not well understood because H\(^+\) and HCO\(_3^-\) transport by duct cells have not been characterized or localized. To address these questions, we microdissected the intralobular, main, and common segments of the rat pancreatic duct. H\(^+\) and HCO\(_3^-\) transporters were characterized and localized by following intracellular pH while perfusing the bath and the lumen of the ducts. In intralobular ducts, Na\(^+\)-dependent and amiloride-sensitive recovery from acid load in the absence of HCO\(_3^-\) was used to localize a Na\(^+/\)H\(^+\) exchanger to the basolateral membrane (BLM). Modification of Cl\(^-\) gradients across the luminal (LM) and BLM in the presence of HCO\(_3^-\) showed the presence of Cl\(^-/\)HCO\(_3^-\) exchangers on both membranes of intralobular duct cells. Measurement of the effect of Cl\(^-\) on one side of the membrane on the rate and extent of pH changes caused by removal and addition of Cl\(^-\) to the opposite side suggested that both exchangers are present in the same cell. In the presence of HCO\(_3^-\), intralobular duct cells used three separate mechanisms to extrude H\(^+\): (a) BLM-located Na\(^+/\)H\(^+\) exchange, (b) Na\(^+\)-independent vacuolar-type H\(^+\) pump, and (c) BLM-located, Na\(^+\)-dependent, amiloride-insensitive, and 4',4'-disothiocyanatostilbene-2,2'-disulfonic acid sensitive mechanism, possibly a Na\(^+\)-dependent HCO\(_3^-\) transporter.

The main and common segments of the duct displayed similar mechanisms and localization of H\(^+\) and HCO\(_3^-\) transporters to the extent studied in the present work. In addition to the transporters found in intralobular ducts, the main and common ducts showed Na\(^+/\)H\(^+\) exchange activity in the LM. Three tests were used to exclude a significant luminal to basolateral Na\(^+\) leak as the cause for an apparent luminal Na\(^+\)/H\(^+\) exchange in an HCO\(_3^-\) secreting cell: (a) addition of amiloride and removal of Na\(^+\) from the LM had a profound effect on Na\(^+\)/H\(^+\) exchange activity on the BLM and vice versa; (b) inhibition of all transporters in the BLM by bathing the duct in the inert hydrocarbon Fluorinert FC-75 did not prevent cytosolic acidification caused by removal of luminal Na\(^+\); and (c) luminal Na\(^+\) did not activate the basolateral Na\(^+\)-dependent HCO\(_3^-\) transporter.

An Na\(^+\)-independent, bafilomycin-sensitive H\(^+\) pumping activity was marginal in the absence of HCO\(_3^-\). Exposing all segments of the duct to CO\(_2\)/HCO\(_3^-\) significantly increased the appearance of H\(^+\) pumping in the plasma membrane. This
effect completely reversed on removal of HCO$_3^-$ from the medium. Stimulation of
the ducts with various agonists in the absence of HCO$_3^-$ also induced H$^+$ pumping
in the plasma membrane. All forms of H$^+$ pump activation were blocked by loading
the duct cells with the Ca$^{2+}$ buffer 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-
tetraacetic acid (BAPTA). Direct measurement of [Ca$^{2+}$]$_i$ showed that HCO$_3^-$ and all
agonists reported to affect pancreatic fluid secretion increased [Ca$^{2+}$]$_i$ in microdis-
sected intralobular ducts and the perfused common ducts. The overall studies
provide the first detailed characterization and localization of H$^+$ and HCO$_3^-$
transporters in the pancreatic duct; report the presence of several new, unsuspected
transporters in the LM and BLM; and should allow better understanding of fluid
and electrolyte secretion by the pancreatic duct.

**INTRODUCTION**

The exocrine pancreas secretes digestive enzymes and Cl$^-$-poor, HCO$_3^-$-rich fluid
(Case, 1989; Kuijpers and De Pont, 1987). Most of the fluid is secreted by the duc
tal tree. The duct also determines the final electrolyte composition of the pancreatic
juice (Shultz, 1987; Case and Argent, 1986). Fluid secretion by the duct requires an
active vectorial transport of Cl$^-$ and HCO$_3^-$ in opposite directions. Some insight into
the molecular mechanisms responsible for secretion by duct cells has been obtained
recently. Isolated intralobular ducts in primary culture were used to demonstrate the
presence of secretin-regulated Cl$^-$ channels in the luminal membrane (LM) (Gray,
Greenwell, and Argent, 1988) and cAMP-regulated maxi K$^+$ channels in the basolat-
eral membrane (BLM) (Gray, Greenwell, Garton, and Argent, 1990). Nonselective
cation channels in the AM and BLM were also found, although their role in fluid
secretion has not been determined (Gray and Argent, 1990). Electrophysiological
studies on microdissected and perfused intralobular ducts showed the presence of a
Ba$^{2+}$-sensitive K$^+$ permeability in the BLM, as well as the effect of amiloride on
membrane potential when added to the BLM, but not the AM of intralobular duct
cells (Novak and Greger, 1988a; Novak and Greger, 1988b). Neither furosemide nor
DIDS added to the BLM had an effect on the electrical properties of duct cells
(Novak and Greger, 1988b). A microspectrofluorometric technique demonstrated the
presence of Na$^+/H^+$ (Stuenkel, Machen, and Williams, 1988; Veel, Villanger, Holthe,
Cragoe, and Raeder, 1992) and Cl$^-$/HCO$_3^-$ (Stuenkel et al., 1988) exchange
mechanisms in intralobular ducts, although the membrane location of the transport-
ers was not determined. All the above studies were performed with intralobular ducts.
Similar studies on the cellular level with other segments of the duct have not been
reported.

Based on the above studies with intralobular ducts from the rat, a model to account
for ductal HCO$_3^-$ and fluid secretion was proposed (Gray et al., 1988; Novak and
Greger 1988b). The model includes a Cl$^-$ channel and a Cl$^-$/HCO$_3^-$ exchanger in
the LM, as well as a K$^+$ channel, Na$^+/H^+$ exchanger, and Na$^+$ pump in the BLM.
HCO$_3^-$ is secreted by the luminal Cl$^-$/HCO$_3^-$ exchanger in exchange for Cl$^-$, which
then leaves the cells through the AM Cl$^-$ channels. The H$^+$ generated in the cytosol
is extruded through the BLM by a Na$^+/H^+$ exchanger, and the Na$^+$ is exchanged for
K$^+$ by the Na$^+$ pump. A variation of this model has been proposed in which H$^+$ efflux
across the BLM is dominated by a vacuolar-type H$^+$ pump and not the Na$^+/H^+$
exchanger (Raeder, 1992). The later model is based on studies showing the presence of acidic vesicles in resting duct cells that disappear on stimulation with secretin (Veel, Buanes, Grotmol, Ostensen, and Raeder, 1991), and the coincidental appearance of BLM foldings when the cells are stimulated (Buanes, Grotmol, Landsverk, and Raeder, 1987; Buanes, Grotmol, Landsverk, Nafstad, and Raeder, 1988).

A general weakness of the proposed models is that $H^+$ and $HCO_3^-$ transporters in pancreatic duct cells have not been thoroughly characterized, let alone localized to the AM or BLM. In addition, micropuncture studies showed dramatic decrease in luminal $Cl^-$ and a reciprocal increase in luminal $HCO_3^-$ along the ductal tree, (i.e., from intralobular to interlobular to main to the common duct) (Lightwood and Reber, 1977; Reber and Wolf, 1968; Case, Harper, and Scratcherd, 1969). This may indicate that different mechanisms mediate $Cl^-$ absorption and $HCO_3^-$ secretion in the different segments of the ductal tree. Hence, in the present studies, we used microdissected intralobular, main, and common ducts, perfused both luminal and basolateral sides, and measured intracellular pH ($pHi$) in the presence and absence of $HCO_3^-$ to characterize and localize the $H^+$ and $HCO_3^-$ transport mechanism in these segments of the ductal tree. We also studied the $CO_2/HCO_3^-$ and agonist-dependent appearance of $H^+$ pump activity in the plasma membrane of duct cells and the role of $[Ca^{2+}]_i$ in this activity. This also required studying the effect of various ductal secretagogues, including secretin and bombesin, on $[Ca^{2+}]_i$.

**METHODS**

**Solutions**

The solutions used in the present experiments were labeled A-G and had the following composition (in mM): solution A (NaCl, Hepes) contained 140 NaCl, 5 KCl, 1 MgCl$_2$, 1 CaCl$_2$, 10 glucose, and 10 Hepes (pH 7.4 with NaOH); solution B (Na$^+$-free, Hepes) was identical to solution A, except that 140 mM N-methyl-D-glucamine (NMG$^+$) Cl$^-$ replaced 140 mM NaCl and pH was adjusted with Tris; solution C (Cl$^-$-free, Hepes) contained 140 Na$^+$-gluconate (Glu), 5 KGlu, 1 MgSO$_4$, 4.6 Ca$^{2+}$-cyclamate, 10 glucose, and 10 Hepes (pH 7.4 with NaOH); solution D (NaCl, $HCO_3^-$) was similar to solution A, except that Hepes was reduced to 2.5 mM, NaCl to 115 mM, and included 25 mM NaHCO$_3$; solution E (Na$^+$-free, $HCO_3^-$) was similar to solution B, except for the reduction of Hepes to 2.5 mM, NMG Cl to 115 mM, and including 25 mM choline-$HCO_3^-$ (this solution also contained 10 $\mu$M atropine to inhibit the cholinergic effect of choline [Muallem, Beeker, and Pandol, 1988]); solution F (Cl$^-$-free, $HCO_3^-$) was similar to solution C, except that Hepes was 2.5 mM, NaGlu was 115 mM, and NaHCO$_3$ was 25 mM; solution G (NaCl-free, $HCO_3^-$) contained 115 MNG$^+$-Glu, 5 KGlut, 1 MgSO$_4$, 4.4 Ca$^{2+}$-cyclamate, 10 glucose, 2.5 Hepes (pH 7.4 with NMGOH), 25 choline$^+$-$HCO_3^-$, and 10 $\mu$M atropine. All solutions containing $HCO_3^-$ were equilibrated with 5% CO$_2$/95% air to pH 7.4 at room temperature. The osmolarity of all solutions was adjusted to 310 mosM with the major salt. Stock solutions of 100 mM amiloride, 100 mM DIDS, 50 mM H$_2$DIDS, 1 mM bafilomycin A1, 2 mM 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF/AM), and 2 mM Fura 2/AM were made in DMSO and stored at $-20^\circ$C.

**Microdissection of Pancreatic Ducts**

Rats weighing 100–200 g were injected with Nembutal and killed by cervical dislocation. After death, an abdominal incision and a V-shaped cut up to the rib cage was made. The skin was cut and removed. A 2 x 2-cm gauze was layered at the bottom of the rib cage. The liver was pulled...
over toward the rib cage and the gauze was folded over the liver. The next stage varied depending on the desired preparation. Fig. 1A shows the entire ductal tree that was microdissected from the pancreas and cleared from acini to show the major parts of the ductal tree that were used in the present studies (see also illustrations in Waynforth, 1988).

FIGURE 1. The experimental preparations used to localize $\text{H}^+$/HCO$_3^-$ transporters in duct cells. The upper panel shows the ductal tree dissected out of a rat pancreas. The posterior and anterior main ducts are connected to the common duct. It is not possible to clearly see the intralobular portion of the duct at the magnification used (~×5). B shows a picture of an in vitro-perfused intralobular duct. The inner perfusion pipette can be seen in the holding pipette and in the lumen. The duct was placed in a perfusion chamber, and the lumen and bath were perfused. The picture shows the dilated lumen of the intralobular duct resulting from the perfusion. The bottom panel shows a portion of the common duct cannulated with a polyethylene tube (C, bottom right). The duct was always cannulated next to its entry to the intestine. The duct was held in place by a plastic holder (C, bottom left). To avoid blockage of flow, a small incision was made next to the holder. The bath perfusion tubing (C, upper right) and the vacuum aspiration outlet (C, upper left) are also shown.

To obtain a perfused common duct, the entry of the duct to the intestine was identified, and the duct was cleared from attached acini and as much as possible connective tissue. One or the two entries of the main duct were ligated to prevent leakage during perfusion. A lateral incision as close as possible to the intestine was made, and a cannula was inserted and ligated. The
cannula used to perfuse the main duct (see Fig. 1 C) was prepared from polyethylene tubing with an inner diameter (I.D.) and outer diameter (O.D.) of 0.48 and 0.64 mm, respectively. After securing the cannula, the duct was cut and removed into a dish containing solution A for dye loading through the lumen.

To obtain a perfused main duct, the common duct was cleaned sufficiently to identify the posterior and anterior entries of the main duct. Then the posterior main duct was cleared from acini and connective tissue and cannulated. The anterior main duct was cleared only if the first cannulation attempt failed. After cleaning, a lateral incision was made and a cannula was inserted. The cannula was inserted as far as possible to form a firm seal of the duct around the cannula. In this case, no ligation was necessary. The cannula was made from a 0.28-mm I.D. and 0.61-mm O.D. polyethylene tubing that was pulled to a tip of 100–120 μm I.D. and 220–240 μm O.D. The final shape of the cannula was conical, allowing the seal of the duct around the cannula.

To facilitate identification of the ductal tree so that intralobular ducts could be dissected, the lumen of the duct was filled with solution A containing several drops of food coloring. For this purpose, the entry of the common duct to the intestine was blocked with a hemostat. An incision was made in the common duct just above the entry of the anterior main duct and a cannula was inserted. About 1 ml of colored solution A was injected to the ductal tree. Injection was continued until dye was seen in intralobular ducts when viewed through a dissecting microscope. Similar staining of the vasculature resulted in different dye distribution and revealed a spiral folding of blood vessels around the ductal tree up to the region of intralobular ducts. The injected pancreas was removed to a cold solution A containing soybean trypsin inhibitor, and it was cut into pieces of ~5–10 mm long. One piece at a time was transferred to a dissecting dish containing the above solution maintained at 4°C. Ducts with stained lumen (and Fura 2 fluorescent when Fura 2/AM was used as described below) were dissected with sharpened forceps. The dye remained restricted to the ducts for ~30 min at 4°C, after which it could be clearly seen in acinar structures.

In some experiments, the pancreas was not cut, but the intralobular duct was identified by gross dissection down the colored ductal tree and was then cleared of acini. The middle panel of Fig. 1 shows how the intralobular duct was microperfused using the technique of Burg (1972) developed to study kidney tubules. The intralobular duct was transferred to a bath chamber constructed of black lucite. The duct was gently aspirated into the holding pipette and the inner perfusion pipette was advanced into the duct (Fig. 1 B, right side). The duct was secured in place by gentle aspiration into a second holding pipette (Fig. 1 B, left side). The intralobular duct lumen was perfused at a flow rate of ~50–100 nl/min. The main duct lumen was perfused at a flow rate of ~100 μl/min, and the common duct lumen was perfused at a flow rate of ~0.7 ml/min. In all experiments, the bath was perfused at a flow rate of 10–12 ml/min.

**Dye Loading**

Preliminary experiments showed that dye loading through the bath should be avoided, particularly with the common and main duct, but also in some segments of intralobular duct. What appeared to be small blood vessels remained attached to some duct segments. The cells in these segments were stained much more intensely than duct cells, especially with BCECF. Furthermore, the connective tissue that remained attached to all duct segments limited dye loading. These problems were solved by loading the dye through the lumen. After establishing luminal and bath perfusion, a small volume of solution A containing BCECF/AM or Fura 2/AM was perfused into the lumen while the bath was continually perfused. The luminal perfusion was stopped for 5–10 min until the fluorescence was between 50 and 200 times above background and then the dyes were washed by perfusing the lumen with solution A.
Since microdissection and perfusion of intralobular ducts were time consuming, to facilitate the \([\text{Ca}^{2+}]\) measurements, these segments were loaded with Fura 2 by an in situ application of luminal Fura 2/AM. About 1 ml of a solution containing 10 \(\mu\text{M}\) Fura 2/AM was infused into the ductal tree through the common duct. After 10 min incubation at room temperature, the lumen was flushed with 10 ml solution A, and then with solution A containing food coloring. The pancreas was then removed into cold solution A for microdissection of intralobular ducts as described above.

After lumen loading of dyes, the fluorescence appeared uniform. Individual cells of the intralobular and the main ducts could be identified, and in these segments, the fluorescence appeared confined to the layer of duct cells. The thick layer of connective tissue prevented the identification of single cells in the common duct, but the fluorescence was uniform when dyes were loaded through the lumen, whereas areas of high fluorescence intensity appeared when dyes were loaded through the bath.

**Measurement of \(pH_i\)**

BCECF fluorescence was recorded from a stretch of 3–12 cells. No differences were noted in \(H^+\) or \(HCO_3^-\) transport based on number of cells used to measure fluorescence. The fluorescence was measured at an emission wavelength of 530 nm with an inverted microscope attached to a dual excitation fluorimeter that provided alternating lights of wavelength 440 and 490 nm as previously described (Muallem, Zhang, Loessberg, and Star, 1992; Star, Zhang, Loessberg, and Muallem, 1992). With the common and main duct, fluorescence was measured with a 40\(\times\) oil immersion objective, whereas with the intralobular duct, either 40\(\times\) or 100\(\times\) oil immersion objectives were used. The emitted light was directed by a dichroic mirror to a photomultiplier tube, and light intensity was measured at a resolution of 2/s. The fluorescence ratios of 490/440 were calibrated intracellularly by perfusing the ducts (lumen and bath) with solutions containing 145 mM KCl, 10 mM Hepes, 5 \(\mu\text{M}\) nigericin, and 10 \(\mu\text{M}\) tri-butyltin with pH adjusted to 6.2–7.6, as previously described (Muallem et al., 1992).

**Measurement of \([\text{Ca}^{2+}]\)**

Two techniques were used to monitor changes in \([\text{Ca}^{2+}]\); (a) photon counting with the setup described above for BCECF (all duct segments), and (b) image acquisition and analysis (microdissected intralobular ducts). The recording system used to obtain the fluorescence images was similar to that used for fluorescence intensity measurements, except that the wavelengths of the excitation light were 340 and 380 nm. The emitted light was directed to a video scope camera, and the signal from eight consecutive frames at each wavelength was integrated before storage. The recording resolution was one set of images (340 and 380) every 6 s. The images were analyzed by a Photon Technology International Imagescan Program. The light or images monitored at 340 and 380 nm were used to calculate the fluorescence intensity or image ratio. The ratios were calibrated to obtain \([\text{Ca}^{2+}]\), as described before (Loessberg, Zhao, Luby-Phelps, Moss, Star, and Muallem, 1993).

**RESULTS**

**Intralobular Ducts**

A major portion of fluid and \(HCO_3^-\) secretion by the stimulated pancreas is mediated by the intralobular portion of the duct (Case, 1989; Case and Argent, 1986). Therefore, to test the models proposed to explain secretion by the pancreatic duct, we measured \(pH_i\) in microdissected and perfused intralobular ducts.
Localization of Na⁺/H⁺ Exchange

Fig. 2 shows that a Na⁺/H⁺ exchange mechanism can be found only in the BLM of the intralobular duct. The experiments were performed with solutions buffered with Hepes to minimize contribution of transporters other than the Na⁺/H⁺ exchanger to pHᵢ regulation (Mualem and Loessberg, 1990a, 1990b). In Hepes-buffered solutions, steady state pHᵢ in all segments of the ducts was similar and averaged 7.36 ± 0.02 (n = 79). Perfusing the lumen of the intralobular duct with a Na⁺-free solution had no effect on pHᵢ, whereas removal of Na⁺ from the bath reduced pHᵢ to ~6.9 (Fig. 2) (average reduction 0.43 ± 0.03 pH units, n = 4). Even when the luminal solution contained Na⁺, removal of Na⁺ from the bath caused a similar reduction in pHᵢ (second portion of Fig. 2). To maximize the rate of H⁺ efflux and to detect the presence of other possible acid transporters, the cytosol was further acidified by a transient exposure of the cells to 20 mM NH₄⁺. On removal of NH₄⁺, pHᵢ was reduced to ~6.5. Addition of Na⁺ to the lumen had no effect on pHᵢ. In contrast,

![Figure 2. Na⁺/H⁺ exchange in the basolateral membrane of the intralobular pancreatic duct. All solutions used in this experiment were buffered with Hepes. The lumen of the intralobular duct was continuously perfused with solution A (NaCl), which was also used to perfuse the bath. Where indicated, the luminal and bath solutions were changed to the Na⁺-free solution B (NMG⁺). To acidify the cytosol, the duct was transiently exposed to NH₄⁺ by replacing 20 mM NMG⁺ with 20 mM NH₄⁺ in the bath-perfusing solution B. To test for the sidedness of Na⁺-dependent H⁺ efflux, the Na⁺-free solution B was replaced by perfusing the lumen and then the bath with the Na⁺-containing solution A. After a second acidification by short incubation with NH₄⁺, the duct was perfused with solution A (NaCl) containing 0.5 mM amiloride. Then amiloride was washed by perfusing the bath with solution A (NaCl).](image)

addition of Na⁺ to the bath resulted in an immediate and rapid increase in pHᵢ. The second portion of the Fig. 2 shows that this effect of Na⁺ could be completely blocked by 0.5 mM amiloride. Thus, the intralobular duct expressed a Na⁺/H⁺ exchange mechanism only in the BLM.

Localization of Cl⁻/HCO₃⁻ Exchange in Intralobular Duct

A Cl⁻/HCO₃⁻ exchanger is believed to be the major HCO₃⁻ efflux mechanism in the luminal membrane (Gray et al., 1990; Novak and Greger, 1988b; Raeder, 1992). This was directly tested by measuring the effect of changing the Cl⁻ gradient across the LM and/or the BLM on pHᵢ. Fig. 3A shows that in the absence of HCO₃⁻, the sequential removal of Cl⁻ from the BLM and the LM acidified rather than alkalinized pHᵢ by ~0.1 pH unit. In the presence of HCO₃⁻, the removal of Cl⁻ from the BLM increased pHᵢ by ~0.15 (0.18 ± 0.02, n = 5) pH units and subsequent removal of HCO₃⁻ from the LM increased pHᵢ to ~7.5 (7.53 ± 0.03, n = 5) (Fig. 3 B). This
finding was unexpected because all models assume that a Cl⁻/HCO₃⁻ exchanger is present only in the LM (Case and Argent, 1986; Gray et al., 1990; Novak and Greger, 1988b; Raeder, 1992; Shultz, 1987). Therefore, we first tested the effect of restoring Cl⁻ to the BLM on pHi. Fig. 3 B shows that addition of Cl⁻ to the BLM resulted in a rapid reduction of pHi from 7.5 to ~7.27 (7.25 ± 0.04, n = 5). Subsequent addition of Cl⁻ to the LM restored the resting pHi.

To further characterize the mechanisms responsible for the Cl⁻-dependent HCO₃⁻ transport in the two membranes, we tested the effect of Na⁺ and DIDS on these activities. Fig. 4 A shows that incubating the luminal and basolateral membranes with an Na⁺-free, HCO₃⁻-containing solution resulted in reduction and stabilization of pHi at ~6.7. Removal of Cl⁻ from the BLM and then the LM, and the subsequent addition of Cl⁻ to the BLM and then the LM, produced the same changes in pHi seen in the presence of Na⁺ (Fig. 3), although at slower rates (Fig. 4 A). This is probably because of the pHi dependency of the Cl⁻/HCO₃⁻ exchanger (Green, Yamaguchi, Kleeman, and Muallem, 1990; Olnes, Tonnessea, Ludt, and Sandrig, 1987). Fig. 4 B shows that treating the duct with 0.5 mM DIDS for ~5 min at 37°C almost completely inhibited the effect of removal of Cl⁻ from the BLM and the LM on pHi.

The experiments in Figs. 3 and 4 suggest that an Na⁺-independent Cl⁻/HCO₃⁻ exchange mechanism exists in both the LM and the BLM of the intralobular pancreatic duct. Fig. 3 B also suggests that the two exchangers in the opposing membranes affect the activity of each other. This is better illustrated in the experiments presented in Fig. 5. In these experiments, we compared the rate and extent of the pHi changes caused by removal and addition of Cl⁻ in the presence and absence of Cl⁻ in the opposite side. Removal of Cl⁻ from the luminal side in the presence of Cl⁻ in the basolateral side slowly increased pHi from ~7.1 to only 7.25. Addition of Cl⁻ to the LM under these conditions slowly restores the initial pHi (not shown). Removal of Cl⁻ from the basolateral side while the lumen was perfused with
a Cl⁻-free solution rapidly increased pHᵢ to ~7.55. Hence, the presence of Cl⁻ in the BLM attenuated the pHᵢ increase caused by the removal of Cl⁻ from the LM. Addition of Cl⁻ to the lumen in the absence of Cl⁻ in the BLM rapidly reduced pHᵢ to ~7.25. However, it is important to note that luminal Cl⁻ did not restore resting pHᵢ and the addition of Cl⁻ to the bath was needed to reduce pHᵢ back to ~7.1. In fact, in all experiments with the same protocol (n = 4 with intralobular ducts, n = 3 with the main duct, and n = 8 with the common duct), addition of Cl⁻ to the lumen only did not restore resting pHᵢ. This provides strong evidence for the operation of a Cl⁻/HCO₃⁻ exchanger in the BLM of the pancreatic duct.

The second portion of Fig. 5 shows that removal of Cl⁻ from the BLM in the presence of Cl⁻ in the LM caused a slow and a small change in pHᵢ. Removal of Cl⁻ from the LM in the absence of Cl⁻ in the BLM resulted in a rapid and large increase in pHᵢ. Thus, the presence of Cl⁻ in the LM attenuated the pHᵢ changes caused by removal of Cl⁻ from the BLM. Finally, in the absence of Cl⁻ in the LM, the addition and removal of Cl⁻ from the BLM caused rapid and large changes in pHᵢ, whereas in the absence of Cl⁻ in the BLM, the addition and removal of Cl⁻ from the LM caused similar rapid and large changes in pHᵢ. The combined protocols in Fig. 5 clearly indicate that the exchanger in each membrane influences the activity of the exchanger in the opposite membrane.

**Na⁺-dependent and -independent H⁺ Efflux**

The experiments in Fig. 2 showed that in Hepes-buffered solutions, an Na⁺/H⁺ exchanger localized to the BLM dominates H⁺ fluxes in intralobular ducts. The
influence of \( \text{HCO}_3^- \) on this exchanger and other \( \text{H}^+ \) and \( \text{HCO}_3^- \) transporters is illustrated in Fig. 6. Exposing the LM and then the BLM to a \( \text{HCO}_3^- \)-buffered solution resulted in the expected transient acidifications. Since the perfusion rate in each side of the duct was sufficiently high, a transient acidification caused by exposure of the BLM and AM to \( \text{CO}_2 \) could be observed. This became a convenient procedure to verify the successful perfusion of the luminal membrane of the duct.

In the presence of \( \text{HCO}_3^- \), \( \text{pH}_i \) stabilized at \( \sim 7.17 \pm 0.02 \) (\( n = 23 \)). Removal of \( \text{Na}^+ \) from the LM of ducts bathed in \( \text{HCO}_3^- \)-buffered solutions had no effect on \( \text{pH}_i \), whereas removal of \( \text{Na}^+ \) from the BLM reduced \( \text{pH}_i \) to \( \sim 6.8 \) (Fig. 6). After an acid load with 20 mM \( \text{NH}_4^+ \) in the absence of \( \text{Na}^+ \) on both membranes, \( \text{pH}_i \) was reduced to \( \sim 6.1 \), but then the ductal cells partially recovered \( \text{pH}_i \), which increased to \( \sim 6.4 \). This was different from the behavior observed in Heps-buffered solution, where little \( \text{Na}^+ \)-independent \( \text{H}^+ \) efflux was observed. The addition of \( \text{Na}^+ \) to the LM had no effect on \( \text{pH}_i \), while addition of \( \text{Na}^+ \) to the BLM resulted in a rapid and complete recovery of \( \text{pH}_i \).
To determine the contribution of the Na\(^+\)/H\(^+\) exchanger to the Na\(^+\)-dependent H\(^+\) efflux, the cells were acidified again in the absence of external Na\(^+\) on both sides of the duct, while including 0.5 mM amiloride in the solution used to wash the NH\(_4\)\(^+\). Amiloride did not prevent the partial recovery of pH\(_i\) after removal of NH\(_4\)\(^+\), excluding the possibility that contaminating Na\(^+\) in intercellular spaces was responsible for this recovery. On the other hand, amiloride attenuated, but did not completely inhibit, H\(^+\) efflux caused by addition of Na\(^+\) to the BLM. Finally, removal of amiloride resulted in recovery of resting pH\(_i\).

The results in Fig. 6 suggest the existence of Na\(^+\)/H\(^+\) exchange and two additional H\(^+\)/HCO\(_3\)\(^-\) transport mechanisms in the presence of HCO\(_3\)\(^-\): (a) an Na\(^+\)-independent H\(^+\) efflux, and (b) an Na\(^+\)-dependent, but amiloride-insensitive pH\(_i\) increase. The nature of these mechanisms have been studied in the common duct, as demonstrated below. To prevent redundancy, similar characterizations are not shown here for the intralobular duct. However, it is clear that the Na\(^+\)-dependent, amiloride-insensitive mechanism is present exclusively in the BLM of the intralobular pancreatic duct.

**MAIN AND COMMON DUCTS**

The HCO\(_3\)\(^-\) and Cl\(^-\) content of the fluid in the main and common portions of the duct is very different from that of the intra- and interlobular ducts (Reber and Wolf, 1968; Case and Argent, 1986). Thus, it was of interest to characterize and localize H\(^+\)/HCO\(_3\)\(^-\) transporters in the main and common ducts. Fig. 1 shows the portions of the main and common ducts used for the purpose of the present studies. These two segments are attached to each other and indeed showed identical properties of pH\(_i\) regulation to the extent tested in the present studies. Most of the studies were performed with the common duct because it could be dissected, cannulated, and perfused easier than the main duct. However, all H\(^+\)/HCO\(_3\)\(^-\) transporters that were identified in the common duct were demonstrated to exist and behave similarly in the main duct. This includes the BLM-located Na\(^+\)/H\(^+\) and Cl\(^-\)/HCO\(_3\)\(^-\) exchangers, the LM-located Cl\(^-\)/HCO\(_3\)\(^-\) exchanger, and the H\(^+\) pump. The most unexpected finding of a Na\(^+\)/H\(^+\) exchanger in the luminal membrane is demonstrated for both ducts.

**Localization of Na\(^+\)/H\(^+\) Exchange**

Fig. 7 shows the presence and relationship of the BLM and LM Na\(^+\)-dependent H\(^+\) efflux mechanisms in the main pancreatic duct. In Hapes-buffered solutions, removal of luminal Na\(^+\) had a small effect on pH\(_i\), while removal of bath Na\(^+\) reduced pH\(_i\) from 7.38 ± 0.03 (n = 3) to 6.83 ± 0.04 (n = 3). After exposure to NH\(_4\)\(^+\) in the absence of Na\(^+\) on both sides of the duct, pH\(_i\) was reduced to ~6.3, and then slightly increased and stabilized at ~6.35. Noticeably, addition of Na\(^+\) to the luminal side rapidly increased pH\(_i\) to ~7.05 ± 0.05 (n = 3). Addition of Na\(^+\) to the bath restored resting pH\(_i\).

The second portion of Fig. 7 shows that removal of Na\(^+\) from the bath in the presence of Na\(^+\) in the lumen had no effect on pH\(_i\). This is very different from the behavior observed in the intralobular duct, where removal of Na\(^+\) from the bath in the presence or absence of luminal Na\(^+\) similarly reduced pH\(_i\) (see Fig. 2). Removal
of luminal Na\(^+\) in the absence of Na\(^+\) in the bath resulted in the expected reduction in pH\(_i\). After acidification by incubation with NH\(_4\)\(^+\), addition of Na\(^+\) to the bath increased pH\(_i\) to \(\sim 7.0 \pm 0.04\) (\(n = 3\)). However, bath Na\(^+\) did not restore resting pH\(_i\). The addition of Na\(^+\) to the luminal solution was required to further increase and restore resting pH\(_i\). All the Na\(^+\)-dependent changes in pH\(_i\) in Hepes-buffered solutions illustrated in Fig. 7 with the main duct were blocked by amiloride (not shown). The amiloride sensitivity will be shown below in the common duct.

Finding a Na\(^+\)-dependent H\(^+\) efflux mechanism in the luminal membrane of a cell secreting HCO\(_3\)\(^-\) was completely unexpected, especially because no such activity was found in the intralobular segment of the ductal tree. An obvious explanation for this observation is that during duct microdissection and cannulation and/or perfusion, the tight junctions were damaged and became leaky to Na\(^+\) ions. Hence, several protocols were used to demonstrate that separate Na\(^+\)-dependent and amiloride-sensitive H\(^+\) efflux activities exist in the LM and BLM of the main and common ducts. The first of these protocols involved comparing the effect of Na\(^+\) removal and inhibition of the Na\(^+\)/H\(^+\) exchanger with amiloride in one membrane on the Na\(^+\)/H\(^+\) exchange in the opposite membrane. The results of these protocols are recorded in Fig. 8. All the experiments in this figure were performed with Hepes-buffered solutions. Fig. 8 A shows that in the presence of luminal Na\(^+\), the removal of Na\(^+\) from the bath resulted in a small reduction in pH\(_i\) that recovered upon adding Na\(^+\) back to the bath. However, in the absence of luminal Na\(^+\), the removal of Na\(^+\) from the bath rapidly and markedly acidified the cytosol. Addition of Na\(^+\) to the bath only partially recovered pH\(_i\), and addition of Na\(^+\) to the lumen further increased pH\(_i\). The subsequent portion of Fig. 8 A shows that adding amiloride to the lumen to inhibit the Na\(^+\)/H\(^+\) exchanger in this membrane was sufficient to expose a cytosolic acidification on removal of Na\(^+\) from the bath. Removal of amiloride from the Na\(^+\)-containing luminal solution in the absence of bath Na\(^+\) resulted in an increase in pH\(_i\). Furthermore, in the presence of bath Na\(^+\) and amiloride, removal of Na\(^+\) from
the lumen was sufficient to trigger cytosolic acidification. Fig. 8B shows the complementary protocol in which removal of bath Na⁺ was required before removal of luminal Na⁺ can cause cytosolic acidification, and that the recovery of pHi of acidified cytosol upon addition of Na⁺ to the lumen could be blocked by amiloride.

A second approach to demonstrating the presence of a luminal Na⁺/H⁺ exchange was to replace the bath solution with the inert fluorinated hydrocarbon Fluorinert FC-75 to prevent all transport events in this membrane (Fig. 9). This procedure was successfully used in localizing transporters in the kidney tubules (Strange and Spring, 1987; Star, 1990). In the control period (not shown), we verified that removal of Na⁺ from the lumen in the presence of Na⁺ in the bath had only a small effect on pHi, similar to that in Fig. 8. Fig. 9 shows that when the bath solution was replaced with Fluorinert FC-75, removal of luminal Na⁺ resulted in cytosolic acidification that was reversed on readdition of Na⁺. The effect was also observed when the luminal solutions contained HCO⁻ and equilibrated with CO₂.

**FIGURE 8. Localization of luminal and basolateral Na⁺/H⁺ exchangers in the common duct.** The two experiments were performed with separate ducts and were designed to show the effect of Na⁺ and/or amiloride in one side on the Na⁺/H⁺ exchange activity in the other side. Thus, all solutions were buffered with Hepes and were either solution A (Na⁺ or Na⁺-free solution B (NMG⁻)). When included, the concentration of amiloride was 0.5 mM. Note that removal of Na⁺ from both sides was required to acidify the cytosol; addition of amiloride to lumen or bath was equivalent to removal of Na⁺ from lumen or bath, respectively, and that addition of amiloride to bath did not prevent acidification caused by removal of Na⁺ from lumen. Finally, addition of amiloride to the lumen in the absence of Na⁺ in bath inhibited the luminal Na⁺-dependent alkalinization (B).

**Na⁺-dependent and -independent H⁺/HCO⁻ Fluxes in the Main and Common Ducts**

Additional evidence for luminal Na⁺/H⁺ exchanger in the common duct was obtained when we studied H⁺/HCO⁻ transport in the presence of HCO⁻. Fig. 10 shows that removal of Na⁺ from one side of the membrane had a minimal effect on pHi of ducts perfused with HCO⁻- buffered solutions. Removal of Na⁺ from the LM or BLM in the absence of Na⁺ in the opposite side resulted in a similar rapid acidification by ~0.47 ± 0.02 (n = 7) pH unit. After acidification by incubation with NH₄⁺ in the presence of HCO⁻, the cells partially recovered pH, which initially acidified to ~6.25 and then stabilized at 6.56 ± 0.03 (n = 32). Fig. 10A shows that perfusing the luminal side with a solution containing Na⁺ and 0.5 mM amiloride had minimal affect on pH. Addition of amiloride to the Na⁺-free bath solution did not
affect pH. However, removal of amiloride from the lumen, while still present in the bath at a concentration sufficient to maximally inhibit BLM Na⁺/H⁺ exchange in the presence of Na⁺ resulted in cytosolic alkalinization. Hence, this experiment shows that an amiloride-sensitive Na⁺-dependent H⁺ efflux is present, and it is the only mechanism of H⁺ efflux in the LM.

The influence of HCO₃⁻ on H⁺/HCO₃⁻ transport in the BLM is shown in Fig. 10 B. In the absence of luminal Na⁺, addition of a solution containing Na⁺, amiloride and H₂DIDS only slightly increased pH. Removal of H₂DIDS in the continuous presence of amiloride resulted in a pH increase close to the resting level. This experiment was possible because the inhibitory effect of H₂DIDS is reversible (Venzl, Sjaastad, Weintraub, and Machen, 1989). Hence, unlike the case in the luminal membrane (Figure 10 A), amiloride alone did not prevent cytosolic alkalinization of previously acidified cytosol. This activity was observed in the presence of HCO₃⁻, and it was blocked by 0.4 mM H₂DIDS (Fig. 10 B) and by 15–20 min of preincubation with 0.5 mM DIDS (not shown).

**Cl⁻/HCO₃⁻ Exchange**

The protocols used to demonstrate the presence of a Cl⁻/HCO₃⁻ exchange mechanism on both membranes of intralobular duct cells were used to show the presence
FIGURE 10. Localization of Na⁺-dependendent HCO₃⁻ transport in the basolateral membrane. The two experiments were performed with the same duct. All solutions in both experiments were buffered with HCO₃⁻. In A, after acidification by replacing solution D with solution A in Na⁺-free medium (NMG⁺), the lumen was perfused with solution D (Na⁺) containing 0.5 mM amiloride. Then the bath was perfused with solution E (NMG⁺) containing 0.5 mM amiloride before removing the amiloride from the lumen. This experiment also shows that there was no significant leak of Na⁺ from the lumen to the bath and all effects of luminal Na⁺ were blocked by amiloride. In B, after the acidification in Na⁺-free solution E (NMG⁺), the bath was exposed to solution D (Na⁺) that also contained 0.5 mM amiloride and 0.4 mM H₂DIDS. Removal of H₂DIDS by perfusing the bath with solution D containing amiloride resulted in pHᵢ increase. Finally, the bath was perfused with solution D.

and examine the behavior of the exchangers in the perfused main and common ducts. Fig. 11 shows the results of these experiments. The major findings are similar to those made with the intralobular duct. Thus, removal of Cl⁻ increased pHᵢ only in the presence but not absence of HCO₃⁻ (not shown). Removal of Cl⁻ from the lumen and then the bath (Fig. 11 A), or bath and then the lumen, (Fig. 11 B) was required...

FIGURE 11. Cl⁻/HCO₃⁻ exchange in the luminal and basolateral membranes of the common duct. All solutions were buffered with HCO₃⁻ and all experiments were performed with the same common duct. The protocol was the same as that in Fig. 5, and it entailed perfusing the luminal and basolateral membranes with solution D (Cl⁻) or Cl⁻-free solution F (Glu⁻). The sequence of Cl⁻ removal and addition to the two sides was alternated in A and B. In C, the luminal and then the basolateral membranes were treated with 0.5 mM DIDS in solution D before exposure to a DISD-containing, Cl⁻-free solution F.
for maximal cytosolic alkalinization. Addition of Cl\textsuperscript{−} to the lumen in the absence of Cl\textsuperscript{−} in the bath (Fig. 11 A) or to the bath in the absence of Cl\textsuperscript{−} in the lumen (Fig. 11 B), was not sufficient to restore resting pH\textsubscript{i}. All the changes in pH\textsubscript{i} caused by changes in cellular Cl\textsuperscript{−} gradient were blocked by DIDS (Fig. 11 C), and they could be observed in the absence of luminal and bath Na\textsuperscript{+} (not shown).

**H\textsuperscript{+} PUMPING**

**Regulation of H\textsuperscript{+} Pumping: Role of CO\textsubscript{2}/HCO\textsubscript{3}−**

While studying the effect of HCO\textsubscript{3}− on the Na\textsuperscript{+} and Cl\textsuperscript{−}-dependent acid/base transporters, we noticed that exposing cells of intralobular ducts, the main or common ducts to CO\textsubscript{2}/HCO\textsubscript{3}−, invariably and significantly increased the Na\textsuperscript{+}-independent recovery from acid load. In many experiments, removal of Na\textsuperscript{+} from both membranes was followed by rapid cellular acidification and subsequent partial recovery of pH\textsubscript{i}. This is illustrated in Fig. 12. In addition, in the absence of Na\textsuperscript{+} and perfused with Na\textsuperscript{+}-free solution E, which resulted in cytosolic acidification to pH\textsubscript{i} of ~6.25 and recovery to pH\textsubscript{i} of ~6.7. The lumen and bath were then perfused with solution D (Na\textsuperscript{+}). After stabilization of pH\textsubscript{i}, the lumen was perfused with Na\textsuperscript{+}-free solution E (NMG\textsuperscript{+}) containing 1 μM bafilomycin. After ~5 min incubation with bafilomycin, the bath solution was changed to solution E. Incubation and removal of 20 mM NH\textsubscript{4}\textsuperscript{+} in the absence of luminal and bath Na\textsuperscript{+} resulted in stable acidification. Finally, the bath and the lumen were perfused with solution D.

The HCO\textsubscript{3}−-induced appearance of H\textsuperscript{+} pumping in the plasma membrane raised the question if this process is dynamic and can be reversed. This was tested in the experiment shown in Fig. 13. After equilibration in HCO\textsubscript{3}−-containing solutions and demonstrating Na\textsuperscript{+}-independent H\textsuperscript{+} efflux, HCO\textsubscript{3}− was removed from the lumen and the bath by perfusing with Heps-buffered solutions. Removal of CO\textsubscript{2}/HCO\textsubscript{3}−-caused pH\textsubscript{i} to increase to ~7.7, which was then stabilized at ~7.35. After removal of Na\textsuperscript{+} from both membranes and acidification by incubation with NH\textsubscript{4}\textsuperscript{+}, the duct cells...
FIGURE 13. Reversal of CO₂/HCO₃⁻-dependent H⁺ pump activation. In the control period, perfusing the bath and the lumen with HCO₃⁻-buffered solutions D (Na⁺) and E (NMG⁺) and transient incubation with 20 mM NH₄⁺ were used to demonstrate Na⁺-independent H⁺ efflux. Then, the lumen and bath solutions were changed to a Hepes-buffered solution A (Hepes, Na⁺). pHᵢ increased because of removal of CO₂. After stabilization of pHᵢ, the same test of H⁺ pump activity was used, except that Hepes-buffered solutions A (Na⁺) and B (NMG⁺) were used.

failed to show Na⁺-independent H⁺ efflux. It is thus clear that incubating the duct in Hepes-buffered solutions for ~20 min (the time required to complete the protocol of transferring the duct from HCO₃⁻ to Hepes and acidifying the cytosol in the absence of Na⁺) was sufficient to reverse the effect of CO₂/HCO₃⁻ on H⁺ pumping.

Regulation of H⁺ Pumping: Role of \([\text{Ca}^{2+}]\),

It was previously reported that an increase in cell \(\text{Ca}^{2+}\) inhibited and delayed activation of H⁺ efflux across the BLM of the outer medullary collecting duct (Hayes and Alpern, 1991). This effect could be prevented by loading the cells with BAPTA (Hayes and Alpern, 1991). Therefore, we attempted to determine whether the same applies to the pancreatic duct. Fig. 14 shows the effect of BAPTA loading on appearance of H⁺ pumping in the pancreatic duct. In the control period, transferring the cells from Hepes to HCO₃⁻-buffered solutions resulted in Na⁺-independent H⁺ efflux, as shown in the first portion of Fig. 14. After stabilization of pHᵢ, the luminal solution was supplemented with 10 μM BAPTA/AM to load the duct cells with BAPTA. Such BAPTA loading completely prevented the CO₂/HCO₃⁻-dependent appearance of H⁺ pumping in the plasma membrane.

Fluid and electrolyte secretion by the pancreatic duct is stimulated by the cAMP stimulating agonist secretin (Ashton, Argent, and Green, 1991) and by the \(\text{Ca}^{2+}\)
mobilizing agonist carbachol (Stuenkel and Hootman, 1990). Previous studies suggested that secretin stimulates the fusion of H⁺ pumps containing intracellular vesicles with the plasma membrane (Veel et al., 1991). Therefore, in the next set of experiments, we determined the effect of the agonists on H⁺ pumping in Hepes-buffered solutions and the role of [Ca²⁺], in these effects. Fig. 15 confirms that in Hepes-buffered solutions, duct cells do not have plasma membrane H⁺ pumping activity. Stimulation of ducts maintained in Hepes-buffered solutions with secretin (trace b₁) or carbachol (trace c₁) before acidification with NH₄⁺ led to the appearance of H⁺ pumping in the plasma membrane. When the same ducts were loaded with BAPTA, both secretin (trace b₂) and carbachol (trace c₂) failed to induce H⁺ pumping in the plasma membrane.

**Figure 15.** Inhibition of agonist-dependent H⁺ pump activation by BAPTA. All solutions were buffered with Hepes and the three experiments were performed with separate ducts. Each figure shows only the portion of the experiment that tested for H⁺ pump activity. The general protocol was to perfuse the lumen and then the bath with a Na⁺-free solution B (NMG⁺) and then to expose the bath to solution B, where 20 mM NH₄⁺ replaced 20 mM NMG⁺. The recovery of pH after removal of NH₄⁺ with solution B (NMG⁺) was used to estimate H⁺ pump activity. After the control period, the lumen and bath were perfused with solution A and the cells were loaded with BAPTA as described in the legend to Fig. 14. After completion of BAPTA loading, H⁺ pump activity was measured in Hepes-buffered solutions as in the control period. Experiments a₁ and a₂ are H⁺ pump activity before (solid line) and after (dashed line) BAPTA loading. In experiments b₁ and b₂, the duct was stimulated with 10 nM secretin for ~10 min before completion of the solution changes needed for measurement of H⁺ pumping. The agonist was removed before BAPTA loading, and the cells were stimulated again with the same concentration of secretin before measurement of H⁺ pump activity in BAPTA-loaded cells. In experiments c₁ and c₂, where indicated, the duct was stimulated with 0.1 mM carbachol before measurement of H⁺ pump activity.

### Effect of HCO₃⁻ and Agonists on [Ca²⁺]

The inhibition of HCO₃⁻ and agonist-mediated H⁺ pump induction by BAPTA suggested that agonists capable of stimulating fluid and electrolyte secretion can also increase [Ca²⁺] of common duct cells. Previous studies showed that carbachol (Stuenkel and Hootman, 1990; Evans, Ashton, Elliott, Green and Argent, 1991) and cholecystokinin (CCK) (Smith, Yelamarty, Kramer, and Cheung, 1993) increase [Ca²⁺], of the cells in microdissected intralobular ducts. In the present studies, we confirm and extended these findings. Fig. 16 shows that in addition to carbachol and CCK, bombesin and secretin also increase [Ca²⁺], of cells in microdissected intralobular ducts. For these experiments, high agonist concentrations were needed to stimulate [Ca²⁺], changes that were oscillatory in nature. [Ca²⁺], oscillations are
Figure 16. Effect of agonists on \([Ca^{2+}]_{i}\) of microdissected intralobular ducts. To avoid signals from cells other than intralobular duct cells, Fura 2 was loaded lumina1ly into all cells of the ductal tree as described under Materials and Methods. Then several segments of intralobular ducts were microdissected and placed in a perfusion chamber. To facilitate experimentation, the lumen of these ducts was not perfused. The bath was perfused with solution A (Hepes-buffered NaCl). Where indicated, the ducts were stimulated with 10 and 100 \(\mu\)M carbachol, 10 nM CCK8, 100 nM bombesin, or 10 nM secretin.

Usually seen at low agonist concentrations (Berridge, 1993). At present, it is not clear why microdissected ducts respond as if the agonists do not have free access to their receptors.

The effect of HCO\(_3^-\) and agonists on \([Ca^{2+}]_{i}\) of cells in the common duct or any perfused duct has not been examined before. To verify that \([Ca^{2+}]_{i}\) changes may be important in modulating H\(^+\) pumping and fluid secretion of these cells, continuously perfused common ducts were used to measure the effect of HCO\(_3^-\) and agonists on \([Ca^{2+}]_{i}\). Fig. 17 shows that all agonists capable of increasing \([Ca^{2+}]_{i}\) in the intralobular duct stimulated a \([Ca^{2+}]_{i}\) increase in cells of the common duct. Figure 17 (bottom) shows that exposing the cells to CO\(_2\)/HCO\(_3^-\) also causes a transient increase in \([Ca^{2+}]_{i}\), further implicating a \([Ca^{2+}]_{i}\) change as the mediator in H\(^+\) pump appearance.

Figure 17. Effect of CO\(_2\)/HCO\(_3^-\) and agonists on \([Ca^{2+}]_{i}\) of perfused common ducts. All experiments were performed in separate ducts. The lumen and bath were perfused with solution A (Hepes-buffered NaCl). Where indicated, the ducts were stimulated with 10 nM secretin, 0.1 mM carbachol, 0.1 \(\mu\)M bombesin, or 10 nM CCK8. To test the effect of CO\(_2\)/HCO\(_3^-\) on \([Ca^{2+}]_{i}\), the lumen and bath perfusates were changed from Hepes to CO\(_2\)/HCO\(_3^-\)-buffered solutions (bottom). After \(~\)10 min incubation and while still in CO\(_2\)/HCO\(_3^-\), the duct was stimulated with 0.1 mM carbachol. Carbachol was added to the bath solution. Similar effects were observed in at least three other experiments.
in the plasma membrane. Interestingly, the CO$_2$/HCO$_3^-$-induced change in [Ca$^{2+}$]$_i$ did not prevent the ability of carbachol to cause a further increase in [Ca$^{2+}$]$_i$. The relationship between HCO$_3^-$ and agonist-dependent [Ca$^{2+}$]$_i$ increases, and H$^+$ pump activation needs to be explored extensively in future studies.

**DISCUSSION**

The bulk of pancreatic fluid and electrolyte secretion is elaborated by the pancreatic ductal system. This process is poorly understood mainly because few studies addressed the nature and membrane localization of the transporters mediating the secretion on the cellular level. The limited studies reported to date used only intralobular ducts. However, it is clear that other portions of the ductal tree must determine the final electrolyte composition of the pancreatic juice because the Cl$^-$ and HCO$_3^-$ content of this fluid is dramatically different when sampled from the intralobular and the main duct (Case and Argent, 1986). Furthermore, the models proposed to explain fluid and electrolyte secretion in intralobular duct (Gray et al., 1988; Novak and Greger, 1988b; Raeder, 1992) are unsatisfactory because they cannot explain several features of fluid secretion. For example, the Cl$^-$/HCO$_3^-$ exchange and the Cl$^-$ shunt pathway provide a mechanism for HCO$_3^-$ secretion but not for net Cl$^-$ absorption. It is doubtful that a Cl$^-$/HCO$_3^-$ exchanger can establish the final concentration of Cl$^-$ and HCO$_3^-$ found in the pancreatic juice. In the rat, the species used in the present studies, the alkaline pancreatic juice contains ~70 mM Cl$^-$ and 80 mM HCO$_3^-$ (Mangos and McSherry, 1971). In other species, such as the guinea pig and man (Padfield, Garner, and Case, 1989; Case and Argent, 1989), HCO$_3^-$ can be as high as 140 mM and Cl$^-$ can be as low as 20 mM. To establish these gradients across the LM of the rat duct by Cl$^-$/HCO$_3^-$ exchange, steady state pH$_i$ during secretion should be ~7.7 or internal Cl$^-$ below 10 mM. In other species, the situation is even more problematic. When tested (Green et al., 1990; Kurtz and Golchini, 1987), HCO$_3^-$ was found to be a competitive inhibitor with external Cl$^-$, and it should stop the exchange before the final concentrations of Cl$^-$ and HCO$_3^-$ in the rat pancreatic juice can be achieved. Hence, it seems necessary to reexamine the mechanism of ductal fluid and electrolyte secretion.

**The H$^+$/HCO$_3^-$ Transporters**

Ion substitution and blocker sensitivities were used to demonstrate the presence of Na$^+$/H$^+$ and Cl$^-$/HCO$_3^-$ exchange, Na-dependent HCO$_3^-$ transport, and a vacuolar-type H$^+$ pump in the plasma membrane of duct cells. These transporters were found in all segments of the duct examined, i.e., intralobular, main, and common parts of the ductal tree. Hence, in the absence of HCO$_3^-$, recovery from acid load required external Na$^+$, and it was inhibited by amiloride. These criteria were used before (Muallem and Loessberg, 1990; Stuenkel et al., 1987) and in the present studies to indicate Na$^+$/H$^+$ exchange activity. Another H$^+$ transporter that was observed only in the presence of HCO$_3^-$ is the H$^+$ pump that was characterized as Na$^+$-independent and bafilomycin blockable cytosolic alkalinization in acid loaded cells (Figure 12).

Na$^+$-independent, DIDS-sensitive, pH$_i$ changes resulting from modification of cellular Cl$^-$ gradient were used to demonstrate Cl$^-$/HCO$_3^-$ exchange in duct cells.
The exchangers in duct cells required HCO₃⁻ and did not accept OH⁻, as was found for the Cl⁻/HCO₃⁻ exchanger in pancreatic acinar cells (Muallem and Loessberg, 1990). This finding can explain why fluid secretion by isolated intralobular ducts required the presence of HCO₃⁻ (Ashton, Argent, and Green, 1990). An Na⁺-dependent HCO₃⁻ transport with properties similar to those found here, which include Na⁺-dependent, amiloride insensitivity, and DIDS sensitivity was reported before in several cell types (Boron and Boulpaep, 1983; Alpern, 1985; Wenzl et al., 1989; Muallem and Loessberg, 1990), and it was attributed to a Na⁺ coupled HCO₃⁻ transport. It is possible that similar coupled cotransporters exist in intralobular (Fig. 6) and common ducts (Fig. 10). However, further studies are needed to exclusively identify this activity.

Localization of the Transporters

A constant finding of the present studies was the BLM localization of the newly described Na⁺-dependent HCO₃⁻ transporter in all segments of the duct. The best evidence for a BLM localization of this transporter was obtained with the intralobular duct because this segment lacked any apparent Na⁺-dependent H⁺ or HCO₃⁻ transporter in the LM (Fig. 6). It was thus possible to show that 0.5 mM amiloride completely blocked recovery from acid load in the absence (Fig. 2), but not in the presence (Fig. 6), of HCO₃⁻. In the case of the main and common ducts (Fig. 10), it was necessary to rely on the extent of inhibition by amiloride of the Na⁺-dependent alkalinization of acidified cytosol to conclude that Na⁺-dependent HCO₃⁻ transport exists in the BLM, but not the LM.

In the presence of HCO₃⁻, all segments of the duct demonstrated H⁺ pump activity. However, we could not determine the sidedness of the pump because bafilomycin was equally effective in blocking the pump when added to the lumen or the bath. Previous studies have shown that stimulation of the ducts with secretin led to disappearance of acid-containing vesicles from the cytoplasm (Veel et al., 1991) and a concomitant large increase in the BLM surface area (Buanes et al., 1987, 1988). This was taken to suggest a BLM localization of the H⁺ pump. Although this is expected to be the case for a HCO₃⁻-secreting epithelia, direct evidence for BLM localization of the H⁺ pump is still required.

Although Cl⁻/HCO₃⁻ exchange activity has been demonstrated in the intralobular duct (Stuenkel et al., 1988), its sidedness has not been determined. The present studies are the first to localize this activity and to show that both membranes of duct cells have a Cl⁻/HCO₃⁻ exchanger. While it was not unexpected to find a luminal Cl⁻/HCO₃⁻ exchange, we did not expect to find an exchange activity in the BLM. Because the pancreatic duct is a Cl⁻-absorbing epithelia and most Cl⁻ transport is transcellular (Case and Argent, 1989), the transepithelial Cl⁻ permeability is low. Indeed, in unstimulated ducts, the contribution of Cl⁻ conductance to LM and transepithelial potentials is marginal (Novak and Greger, 1988a, 1991). This makes it unlikely that a Cl⁻ leak from the lumen to bath or from bath to lumen was responsible for the apparent Cl⁻/HCO₃⁻ exchange in the BLM. Furthermore, removal of Cl⁻ from the bath only always increased pHᵢ, and addition of Cl⁻ to the lumen in the absence of Cl⁻ in the bath never reduced pHᵢ to resting levels (Figs. 3–5 and 11).
Cl⁻/HCO₃⁻ exchange in the luminal and BLM was found in all segments of the duct. Furthermore, the experiment in Fig. 5 using intralobular ducts, and similar experiments performed with the main and common ducts (not shown), suggest that the two exchangers exist in the same or in H⁺/HCO₃⁻-coupled neighboring cells. In the case of the intralobular and main ducts, single cells could be identified and used for single-cell recording to localize the exchangers to the same cell. However, duct cells are relatively small and closely packed, and BCECF has a high quantum yield so that it was not possible to exclude the contribution of neighboring, uncoupled cells to the recorded fluorescence. A complementary approach to single-cell recording was to determine the rate and extent of alkalinization caused by the sequential removal of Cl⁻ from the two membranes. If the BLM and LM exchangers were present on separate uncoupled cells, the sequence of Cl⁻ removal should have no effect on the extent of the pHᵢ increase. Only if the exchangers exist in the same or coupled cells and affect the activity of each other should the sequence of Cl⁻ removal determine the extent of alkalinization. Indeed, removal of Cl⁻ from each side had a markedly different effect, depending on whether Cl⁻ was present or absent from the other side of the duct (Fig. 5).

An Na⁺/H⁺ exchange activity in intralobular ducts has been reported (Stuenkel et al., 1988; Veel et al., 1992). Furthermore, addition of amiloride to the BLM, but not the LM, depolarized the membrane potential of intralobular duct cells (Novak and Greger, 1988a). This was interpreted as inhibition of a BLM-located Na⁺/H⁺ exchanger that acidified the cell, and as a consequence inhibited K⁺ channels (Novak and Greger, 1988a). In the present studies, we provide direct evidence for the BLM localization of the Na⁺/H⁺ exchanger in intralobular duct. We also show the presence of the changer in the BLM of the main and common duct cells. In addition, we did not find any Na⁺-dependent H⁺ or HCO₃⁻ transport in the luminal membrane of the intralobular duct.

A completely unexpected finding was an Na⁺/H⁺ exchange activity in the luminal membrane of the main and common ducts, but not in intralobular ducts. An apical Na⁺/H⁺ exchanger seems out of place in a portion of the duct where HCO₃⁻ concentration is the highest during stimulation of fluid secretion. Because most transductal Na⁺ transport is believed to be paracellular, and the tight junctions of duct cells are permeable to Na⁺ (Case and Scratcherd, 1974; Kuijpers, De Pont, and Westerhoff, 1989), a possible explanation for an apparent luminal Na⁺/H⁺ exchange that had to be considered is high luminal to basolateral Na⁺ leak. Hence, we sought to develop independent evidence to verify the presence of a luminal Na⁺/H⁺ exchange activity. The first approach was to prevent Na⁺ from reaching the BLM and to inhibit all transporters at this membrane by bathing the duct in Fluorinert FC-75. Fluorinert FC-75 also blocks any fluid flow from the LM to the BLM. Bathing the duct in Fluorinert F-75 did not prevent the acidification and subsequent alkalinization in the presence or absence of HCO₃⁻, initiated by removal and addition of luminal Na⁺, respectively.

The second approach was to show that addition of amiloride to the LM was equivalent to Na⁺ removal from the lumen (Fig. 8 A). Thus, removal of Na⁺ from the LM always resulted in a small cytosolic acidification. If the LM has no Na⁺/H⁺ exchange, this can be the case if bath Na⁺ poorly accesses the BLM. This does not
appear to be the case because in the absence of luminal Na⁺, removal and addition of Na⁺ to the bath solution resulted in rapid and large pHᵢ changes. Addition of amiloride to the luminal solution acidified the cytosol similar to removal of luminal Na⁺ in the presence of bath Na⁺. If the luminal membrane has no Na⁺/H⁺ exchange, this is possible only if amiloride was freely permeable through the tight junctions and reached a concentration of 0.5 mM at the BLM in the face of continuous perfusion with an amiloride-free solution. In this case, removal of Na⁺ from the bath in the continuous presence of amiloride in the lumen should have resulted with slow and small cytosolic acidification. This was not the case. In addition, if the Na⁺/H⁺ exchanger was present only in the BLM, then including amiloride only in the bath solution should have been sufficient to acidify the cytosol similar to removal of Na⁺ from the BLM while including Na⁺ and amiloride in the LM. Addition of amiloride only to the bath acidified the cytosol to the same extent as the addition of amiloride only to the lumen.

The last evidence for a luminal Na⁺/H⁺ exchange came from testing the effect of HCO₃⁻ on recovery from acid load (Fig. 10A). In these experiments, addition of Na⁺ and amiloride to the lumen had no effect on pHᵢ. If the tight junctions were permeable to Na⁺, then pHᵢ should have increased because of Na⁺-dependent HCO₃⁻ transport present in the BLM and not inhibitable by addition of amiloride to this membrane (see Fig. 10B). Finally, including amiloride in the Na⁺-free bath solution did not prevent the recovery from acid load upon removal of amiloride from the Na⁺-containing luminal solution.

The evidence in Figs. 8–10 forces us to conclude that the luminal membrane of the main and common ducts has an amiloride-sensitive, Na⁺-dependent H⁺ efflux, and thus, Na⁺/H⁺ exchange activity.

**Regulation of H⁺ Pumping**

Exposure of all ductal segments to solutions containing CO₂/HCO₃⁻ always resulted in appearance in the plasma membrane of a H⁺ pump activity. These findings are reminiscent of those reported in the turtle urinary bladder (Gluck, Cannon, and Al-Awqati, 1982; Cannon, van Adelsberg, Kelly, and Al-Awqati, 1985; van Adelsberg and Al-Awqati, 1986) and the proximal and collecting tubules of the kidney (Schwartz and Al-Awqati, 1985), where exposure of the cells to solutions containing CO₂/HCO₃⁻ led to insertion of H⁺ pumps in the plasma membrane. In the kidney and turtle bladder, the pumps were inserted in the luminal membrane. If H⁺ pump insertion in the plasma membrane was responsible for the appearance of H⁺ pump in the pancreatic duct, it is more likely that the H⁺ pumps were inserted in the BLM. Since the duct is a HCO₃⁻-secreting epithelia, a BLM insertion will be of physiological significance (Raeder, 1992). Furthermore, this can explain the appearance of foldings in the BLM with stimulation of ductal secretion (Buanes et al., 1987, 1988).

Stimulation of the ducts with secretin and carbachol also increased H⁺ pumping. A common property for all mode of stimulation is that they could be prevented by loading the ducts with BAPTA to buffer any changes in [Ca²⁺]. In this respect, the insertion of H⁺ pumps by CO₂/HCO₃⁻ in the turtle bladder was preceded by an increase in [Ca²⁺], and could be prevented by loading the cytosol with Ca²⁺ chelators (Cannon et al., 1985; van Adelsberg and Al-Awqati, 1986). Thus, it is possible that all
forms of H+ pump insertion in the pancreatic duct are mediated by an increase in [Ca2+]i. Further support for this notion was obtained when we found that CO2/HCO3- and all agonists known to affect pancreatic (Gardner and Jensen, 1986; Hootman and Williams, 1987) or ductal secretion (Case and Argent, 1989) increased [Ca2+]i in intralobular, main, and common ducts. This includes an effect of secretin and bombesin on [Ca2+]i. Previous studies reported the effect of carbachol (Stuenkel and Hootman, 1990; Evans et al., 1991) and CCK (Smith et al., 1993) on [Ca2+]i of intralobular duct cells. However, an effect of secretin and bombesin could not be demonstrated (Stuenkel and Hootman, 1990; Evans et al., 1991). The reason for the differences in the two studies are not clear. Lack of luminal perfusion cannot be the reason because the agonist similarly affected [Ca2+]i of luminally perfused (Fig. 17) and nonperfused (Fig. 16) ducts. However, previous studies were performed with isolated duct cells maintained in culture for 1 wk (Stuenkel and Hootman, 1990) or with intralobular duct segments maintained in culture for 24–48 h (Ashton et al., 1991). In both cases, the ducts were subjected for some time to collagenase digestion. This may have affected the [Ca2+]i signaling.

Additional conclusions from our findings is that pancreatic ductal secretion may be simultaneously regulated by [Ca2+]i and cAMP. Secretin is a major secretagogue of ductal fluid and electrolyte secretion (Case and Argent, 1989; Raeder, 1992). It increases cellular cAMP (Case, Johnson, Scratcherd, and Sherratt, 1972; Arkle, Lee, Cullen, and Argent, 1986), as well as the activity of the cAMP activates Cl- channels (Gray et al., 1988; Gray, Plant, and Argent, 1993) in the luminal membrane, which is essential for ductal secretion (Kopelman, Durie, Gaskin, Weizman, and Forstner, 1985; Ashton et al., 1991). On the other hand, the present studies show that secretin also stimulates an increase in [Ca2+]i, that may be in the form of [Ca2+]i oscillations (Fig. 16). The changes in [Ca2+]i may stimulate insertion of H+ pumps in the BLM. Studies in vivo suggest that H+ pump–mediated H+ efflux is required for ductal fluid secretion (Grotmol, Buanes, and Raeder, 1986; Veel et al., 1992). Thus, the two signaling systems activated by secretin may be used to control events in separate membranes, Cl- channels in the LM (cAMP) and H+ pumping in the BLM (Ca2+).

**Physiological Significance**

Three transporters localized to the BLM can participate in H+ efflux (Na+/H+ exchanger and H+ pump) or HCO3- influx (Na+-dependent HCO3- transport) during HCO3- secretion across the apical membrane of the duct. Based on transport rates measured in acid-loaded isolated ducts, it seems that the Na+/H+ exchanger dominates H+ efflux across the BLM. If these differences in H+ transport rates and capacities can be extended to physiological condition, then the Na+/H+ exchange should mediate most H+ efflux during stimulation of HCO3- secretion. However, on the bases of their measurements of ductal secretion in vivo and Na+/H+ exchange rates in vitro, Raeder and co-workers (Veel et al., 1992; Raeder, 1992) argued against a significant role for the Na+/H+ exchanger in ductal fluid secretion. One of their major arguments is that maximal rate of H+ efflux by the exchanger in isolated ducts can remove only a small percent of the cytosolic H+ generated during HCO3- secretion by the intact pancreas (Veel et al., 1992). This seems a problematic argument because, in isolated ducts, the rate of H+ efflux by H+ pumps is lower than
that by the exchanger, and thus should be less likely to mediate BLM H⁺ efflux during ductal HCO₃⁻ secretion. On the other hand, as a primary active mechanism, the pump can support a steeper H⁺ gradient than a secondary active transport such as the Na⁺/H⁺ exchanger. Therefore, it is possible that in intralobular ducts, the exchanger mediate most of H⁺ efflux. As the fluid in the duct becomes more alkaline and rich in HCO₃⁻, the role of the H⁺ pump in H⁺ efflux across the BLM increases.

The Na⁺-dependent HCO₃⁻ transport may play a modulatory role in ductal fluid secretion. As an electrogenic process (Boron and Boulpaep, 1983; Muallem and Loessberg, 1990a), it is sensitive to the membrane potential (Boron and Boulpaep, 1983). As discussed before for other cell types (Alpern, 1985; Boron and Boulpaep, 1983; Muallem and Loessberg, 1990a), in resting cells, when the membrane potential is between −60 and −80 mV (Novak and Greger, 1988a, 1991), the cotransporter can mediate HCO₃⁻ efflux across the BLM. On stimulation, the membrane potential of rat duct cells depolarizes to ~−40 to −20 mV (Novak and Greger, 1988b, 1991) where the cotransporter can mediate HCO₃⁻ influx. Such an influx, together with Na⁺/H⁺ exchange, will alkalinize the cytosol and stimulate the Cl⁻/HCO₃⁻ exchanger at the LM. Indeed, during stimulation of H⁺ secretion by the parietal cells, the Na⁺/H⁺ exchanger is activated to increase pHᵢ, which is required for activation of Cl⁻/HCO₃⁻ exchange (Muallem, Blissard, Crago, and Sachs, 1988).

The only HCO₃⁻ efflux mechanism in the LM identified in the present studies is the Cl⁻/HCO₃⁻ exchanger. This exchanger probably mediates most of HCO₃⁻ efflux in intralobular ducts. As argued in the beginning of this discussion, it is difficult to explain HCO₃⁻ efflux in the main and common ducts by an electroneutral, 1:1 Cl⁻/HCO₃⁻ exchange. It is possible that other HCO₃⁻ efflux mechanisms in the LM of the main duct were not found because the ducts were perfused with solutions containing 25 mM HCO₃⁻, pH 7.4, and were not stimulated. During stimulation of secretion, the lumen is exposed to ~80 mM HCO₃⁻, which by itself may activate additional mechanism. Addressing this problem needs further studies.

The two transporters that are not likely to be active during ductal HCO₃⁻ secretion are the BLM Cl⁻/HCO₃⁻ exchanger and the luminal Na⁺/H⁺ exchanger found in distal portions of the pancreatic duct. Obviously, their physiological role is not known at present. One function can be a housekeeping role, analogous to the case in the kidney proximal tubule where luminal Na⁺/H⁺ exchanger is responsible for H⁺ secretion, and BLM exchanger is believed to be responsible for regulation of pHᵢ. In this case, one would expect to find luminal Na⁺/H⁺ exchangers in all segments of the duct. The fact that luminal exchanger is found only in the distal portion of the ductal tree leads us to speculate that these transporters may be important during resting periods in keeping the duct lumen acidic. Duct cells not only secrete, but also appear to respond to CO₂/HCO₃⁻ by activation/insertion of ion transporters needed for HCO₃⁻ secretion, such as K⁺ channels (Novak and Greger, 1991) and H⁺ pumps (present studies). It is possible that duct cells are sensitive to luminal CO₂/HCO₃⁻ as a form of positive feedback mechanisms. In this case, it will be advantageous or even necessary for the ducts to keep the lumen acidic by performing net H⁺ efflux when acinar cells do not secrete digestive enzymes to prevent uncontrolled HCO₃⁻ and fluid secretion. The luminal Na⁺/H⁺ exchanger can perform this task. Since the fluid
in the main and common ducts contain the highest HCO$_3^-$ concentration, it is not unexpected that this segment and not the intralobular duct has the capacity to secrete acid to the lumen.

In summary, the present studies identified and localized the major H$^+$ and HCO$_3^-$ transporters in the pancreatic duct and described some regulatory features of the H$^+$ pump. Although these studies are not sufficient to firmly support a specific mechanism of ductal HCO$_3^-$ and fluid secretion, they can be a framework for future studies of this important physiological activity of the pancreas.

References

Alpern, B. J. 1985. Mechanism of basolateral membrane H$^+$/OH$^-$/HCO$_3^-$ transport in the rat proximal convoluted tubule. Journal of General Physiology. 86:613-636.

Arkle, S., C. M. Lee, M. J. Cullen, and B. E. Argent. 1986. Isolation of ducts from the pancreas of copper-deficient rats. Quarterly Journal of Experimental Physiology. 71:249-265.

Ashton, N., B. E. Argent, and R. Green. 1990. Effect of vasoactive intestinal peptide, bombesin and substance P on fluid secretion by isolated rat pancreatic ducts. Journal of Physiology (London). 427:471-482.

Ashton, N., B. E. Argent, and R. Green. 1991. Characteristics of fluid secretion from isolated rat pancreatic ducts stimulated with secretin and bombesin. Journal of Physiology (London) 435:533-546.

Berridge, M. J. 1993. Inositol trisphosphate and calcium signalling. Nature. 361:315-325.

Boron, W. F., and E. L. Boulpaep. 1983. Intracellular pH regulation in the renal proximal tubule of the sulamander. Journal of General Physiology. 81:55-94.

Buanes, T., T. Grotmol, T. Landsverk, M. G. Raeder. 1987. Ultrastructure of pancreatic duct cells at secretory rest and during secretin-dependent NaHCO$_3^-$ secretion. Acta Physiologica Scandinavica. 131:55-62.

Buanes, T., T. Grotmol, T. Landsverk, P. Nafstad, M. G. Raeder. 1988. Effects of arterial pH and carbon dioxide on pancreatic exocrine H$^+$/HCO$_3^-$ secretion and secretin-dependent translocation of cytosplasmic vesicles in pancreatic duct cells. Acta Physiologica Scandinavica. 133:1-9.

Burg, M. B. 1972. Single tubule perfusion technique. Yale Journal of Biological Medicine. 45:321-328.

Cannon, D., J. van Adelsberg, S. Kelly, and Q. Al-Awqati. 1985. Carbon-dioxide-induced exocytic insertion of H$^+$ pumps in turtle-bladder luminal membrane: role of cell pH and calcium. Nature. 314:443-446.

Case, R. M., A. A. Harper, and T. Scratcherd. 1969. The secretion of electrolytes and enzymes by the pancreas of the anaesthetized cat. Journal of Physiology. 201:335-348.

Case, R. M., M. Johnson, T. Scratcherd, and H. S. A. Sherratt. 1972. Cyclic adenosine 3',5'-monophosphate concentration in the pancreas following stimulation by secretin, cholecystokin-in-pancreozymin and acetylcholine. Journal of Physiology. 223:669-684.

Case, R. M., and T. Scratcherd. 1974. The secretion of alkali metal ions by the perfused cat pancreas as influenced by the composition and osmolality of the external environment and by inhibitors of metabolism and Na$^+$.K$^+$-ATPase activity. Journal of Physiology. 242:415-428.

Case, M. R., and B. E. Argent. 1986. Bicarbonate secretion by pancreatic duct cells—mechanisms and control. In The Exocrine Pancreas: Biology, Pathobiology and Diseases. Ed: V. L. Go, et al, Raven Press, New York pp. 215-242.

Case, M. R. 1989. Physiology and biochemistry of pancreatic exocrine secretion. Current Opinions in Gastroenterology. 5:665-681.
Case, R. M., B. E. Argent. 1989. Pancreatic secretion of electrolytes and water. In Schultz S. G. Forte, J. G., Fauner, B. B., eds. Handbook of physiology. The gastrointestinal system. Volume 3. New York: Oxford University 383–417.

Evans, R. L., N. Ashton, A. C. Elliott, R. Green, and B. E. Argent. 1991. A calcium pathway for activation of fluid secretion by pancreatic ducts. Pediatric Pulmonology. 11(Supplement 6):253–254.

Gardner, J. D. and R. T. Jensen. 1986. Receptors and cell activation associated with pancreatic enzyme secretion. Annual Review of Physiology. 48:103–117.

Gluck, S., C. Cannon, and Q. Al-Aweqati. 1982. Exocytosis regulates urinary acidification in turtle bladder by rapid insertion of H+ pumps into the luminal membrane. Proceedings of the National Academy of Science, USA. 79:4327–4331.

Gray, M. A., J. R. Greenwell, and B. E. Argent. 1988. Secretin-regulated chloride channel on the apical plasma membrane of pancreatic duct cells. Journal of Membrane Biology. 105:131–142.

Gray, M. A., J. R. Greenwell, A. J. Garton, and B. E. Argent. 1990. Regulation of Maxi-K+ channels on pancreatic duct cells by cyclic AMP-dependent phosphorylation. Journal of Membrane Biology. 115:203–215.

Gray, M. A., and B. E. Argent. 1990. Non-selective cation channel on pancreatic duct cells. Biochimica et Biophysica Acta. 1029:547–554.

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 Ca2+ ions. Journal of General Physiology. 95:121–145.

Grotmol, T., T. Buanes, and M. G. Raeder. 1986. N,N'-Dicyclohexylcarbo-diimide (DCCD) reduces pancreatic NaHCO3 secretion but not pancreatic ATP levels. Acta Physiologica Scandinavica. 128:547–554.

Hayes, S. R., and R. J. Alpern. 1991. Inhibition of Na+-independent H+ pump by Na+-dependent changes in cell Ca2+. Journal of General Physiology. 98:781–803.

Hootman, S. R., and J. A. Williams. 1987. Stimulus-secretion coupling in the pancreatic acinus. In Physiology of the Gastrointestinal Tract. Ed: L. R. Johnson, Raven Press, N.Y. pp. 1129–1146.

Kopelman, H., P. Durie, K. Gaskin, A. Weizman, and G. Forstner. 1985. Pancreatic fluid secretion and protein hyperconcentration in cystic fibrosis. New England Journal of Medicine. 312:329–334.

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

Kuijpers, G. A. J., J. J. H. M. De Pont, and H. V. Westerhoff. 1989. A model for fluid secretion in the exocrine pancreas. Biochimica et Biophysica Acta. 984:71–80.

Kurtz, I., and K. Golchini. 1987. Na+-independent Cl–HCO3− exchange in Madin-Darby Canine kidney cells. Role in intracellular pH regulation. Journal of Biological Chemistry. 262:4516–4520.

Lightwood, R., and H. A. Reber. 1977. Micropuncture study of pancreatic secretion in the cat. Gastroenterology. 72:61–66.

Loessberg, P. A., H. Zhao, K. Luby-Phelps, R. L. Moss, R. A. Star, and S. Muallem. 1993. Gap junction communication modulates [Ca2+], oscillations and enzyme secretion in pancreatic acini. Journal of Biological Chemistry. 268:19769–19775.

Mangos, J. A., and N. R. McSherry. 1971. Micropuncture study of excretion of water and electrolytes by the pancreas. American Journal of Physiology. 221:496–503.

Muallem, S., T. Beeker, and S. J. Pandol. 1988. The role of Na+/Ca2+ exchange and the plasma membrane Ca2+ pump in hormone mediated Ca2+ efflux from pancreatic acini. Journal of Membrane Biology. 102:153–162.
Muallem, S., D. Blissard, E. J. Crago, and G. Sachs. 1988. Activation of the Na+/H+ and Cl−/HCO3− exchange by stimulation of acid secretion in the parietal cell. Journal of Biological Chemistry. 263:14703–14711.

Muallem, S. and P. A. Loessberg. 1990a. Intracellular pH-regulatory mechanisms in pancreatic acinar cells. I. Characterization of H+ and HCO3− transporters. Journal of Biological Chemistry. 265:12806–12812.

Muallem, S. and P. A. Loessberg. 1990b. Intracellular pH-regulatory mechanism in pancreatic acinar cells. II: Regulation of H+ and HCO3− transporters by Ca2+-mobilizing agonists. Journal of Biological Chemistry. 265:12813–12819.

Muallem, S., B.-X. Zhang, P. A. Loessberg, and R. A. Star. 1992. Simultaneous recording of cell volume changes and intracellular pH or Ca2+ concentration in single osteosarcoma cells UMR-106-01. Journal of Biological Chemistry. 267:17664–17658.

Novak, I. and R. Greger. 1988a. Electrophysiological study of transport systems in isolated perfused pancreatic ducts: Properties of the basolateral membrane. Pfluegers Archives. 411:58–68.

Novak, I. and R. Greger. 1988b. Properties of the luminal membrane of isolated perfused rat pancreatic ducts. Pfluegers Archives. 411:58–68.

Novak, I. and R. Greger. 1991. Effect of bicarbonate on potassium conductance of isolated perfused rat pancreatic ducts. Pfluegers Archives. 419:76–83.

Ollis, S., T. I. Tonnesea, J. Ludt, and K. Sandrig. 1987. Effect of intracellular pH on the rat of chloride uptake and efflux in different mammalian cell lines. Biochemistry. 26:2778–2785.

Padfield, P. J., A. Garner, and M. R. Case. 1989. Patterns of pancreatic secretion in the anaesthetized guinea pig following stimulation with secretin, cholecystokinin octapeptide and bombesin. Pancreas. 4:204–209.

Raeder, M. G. 1992. The origin of and subcellular mechanisms causing pancreatic bicarbonate secretion. Gastroenterology. 103:1674–1684.

Reber, H. A., and C. J. Wolf. 1968. Micropuncture of pancreatic electrolyte secretion. American Journal of Physiology. 215:34–40.

Schwartz, C. J., and Q. Al-Awqati. 1985. Carbon dioxide causes exocytosis of vesicles containing H+ pumps in isolated perfused proximal and collecting tubules. Journal of Clinical Investigation. 75:1638–1644.

Shultz, I. 1987. Electrolyte and fluid secretion in the exocrine pancreas. In Physiology of the Gastrointestinal Tract. Ed. L. R. Johnson, Raven Press, N.Y., pp. 1147–1171.

Smith, J. P., R. V. Yelamarty, S. T. Kramer, and Y. Cheung. 1993. Effects of cholecystokinin on cytosolic calcium in pancreatic duct segments. American Journal of Physiology. 264:G1177–G1183.

Star, R. A. 1990. Apical membrane limit urea permeation across the rat inner medulary collecting duct. Journal of Clinical Investigation. 86:1172–1178.

Star, R. A., B.-X. Zhang, P. A. Loessberg, and S. Muallem. 1992. Regulatory volume decrease in the presence of HCO3− by single osteosarcoma cells UMR-106-01. Journal of Biological Chemistry. 267:17665–17669 (App. 5).

Strange, K., and K. K. Spring. 1987. Cell membrane water permeability of rabbit cortical collecting ducts. Journal of Membrane Biology. 96:27–43.

Stuenkel, E. L., T. E. Machen, and J. A. Williams. 1988. pH regulatory mechanism in rat pancreatic ductal cells. American Journal of Physiology. 254:G925–G930.

Stuenkel, E. L., and S. R. Hootman. 1990. Secretagogue effects on intracellular calcium in pancreatic duct cells. Pfluegers Archives. 416:652–658.

van Adelsberg, J., and Q. Al-Awqati. 1986. Regulation of cell pH by Ca2+–mediated insertion of H+–ATPase. Journal of Cell Biology. 103:1638–1645.
Veel, T., T. Buanes, T. Grotmol, J. Ostensen, and M. G. Raeder. 1991. Secretin dissipates red acridine orange fluorescence from pancreatic duct epithelium. *Acta Physiologica Scandinavica*. 141:221–226.

Veel, T., O. Villanger, M. S. Holthe, E. J. Cragoe, and M. G. Raeder. 1992. Na\(^+\)-H\(^+\) exchange is not important for secretin-dependent pancreatic HCO\(_3\)\(^-\) secretion in the pig. *Acta Physiologica Scandinavica*. 144:239–246.

Waynforth, H. B. 1988. Experimental and surgical technique in the rat. Academic Press, London, pp. 127–130.

Wenzl, E., M. D. Sjaastad, W. H. Weintraub, and T. E. Machen. 1989. Intracellular pH regulation in IEC-6 cells, a crypt-like intestinal cell line. *American Journal of Physiology*. 267:G732–G740.