CO_2 permeability and bicarbonate transport in microperfused interlobular ducts isolated from guinea-pig pancreas

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1. Permeabilities of the luminal and basolateral membranes of pancreatic duct cells to CO_2 and HCO_3^- were examined in interlobular duct segments isolated from guinea-pig pancreas. Intracellular pH (pH_i) was measured by microfluorometry in unstimulated, microperfused ducts loaded with the pH-sensitive fluoroprobe 2′7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF).

2. When HCO_3^-/CO_2 was admitted to the bath, pH_i decreased transiently as a result of CO_2 diffusion and then increased to a higher value as a result of HCO_3^- uptake across the basolateral membrane by Na^+–HCO_3^- cotransport.

3. When HCO_3^-/CO_2 was admitted to the lumen, pH_i again decreased but no subsequent increase was observed, indicating that the luminal membrane was permeable to CO_2 but did not allow HCO_3^- entry to the cells from the lumen. Only when the luminal HCO_3^- concentration was raised above 125 mM was HCO_3^- entry detected. The same was true of duct cells stimulated with forskolin.

4. Recovery of pH_i from an acid load, induced by exposure to an NH_4^+ pulse, was dependent on basolateral but not luminal Na^+ and could be blocked by basolateral application of methylisobutylamiloride and H_2DIDS. This indicates that the Na^+–H^+ exchangers and Na^+–HCO_3^- cotransporters are located exclusively at the basolateral membrane.

5. In the presence of HCO_3^-/CO_2, substitution of basolateral Cl^- with glucuronate caused larger increases in pH_i than substitution of luminal Cl^-. This suggests that the anion exchanger activity in the basolateral membrane is greater than that in the luminal membrane.

6. We conclude that the luminal and basolateral membranes are both freely permeable to CO_2, but while the basolateral membrane has both uptake and efflux pathways for HCO_3^-, the luminal membrane presents a significant barrier to the re-entry of secreted HCO_3^-, largely through the inhibition of the luminal anion exchanger by high luminal HCO_3^- concentrations.

The ductal system of the exocrine pancreas produces a bicarbonate-rich fluid secretion in response to secretin and some other stimuli. However, there are significant species-dependent variations in the pattern of pancreatic HCO_3^- secretion *in vivo*. In the guinea-pig (Padfield *et al.* 1989) and in several other species, including dog, cat and human (Case & Argent, 1993), the HCO_3^- concentration of the juice may reach 140–150 mM during maximal stimulation with secretin. In the rat, however, the highest HCO_3^- concentration is about 70 mM (Sewell & Young, 1975). We have recently reported that interlobular duct segments isolated from guinea-pig pancreas secrete a HCO_3^--rich fluid (> 130 mM) during stimulation with 10 nM secretin (Ishiguro *et al.* 1998), which indicates that the *in vivo* behaviour of pancreatic ducts is well preserved in this *in vitro* preparation.

In order to maintain such a high concentration of HCO_3^- in pancreatic juice during sustained secretion, pancreatic duct cells must possess the following characteristics. (1) HCO_3^- must be actively accumulated in the cell by transport across the basolateral membrane. We have previously shown that Na^+–HCO_3^- cotransport plays a dominant role in this process (Ishiguro *et al.* 1996*a*). (2) The luminal membrane must possess a mechanism which specifically transports HCO_3^- into the lumen. In a previous paper (Ishiguro *et al.* 1996*b*), we demonstrated a secretin-stimulated HCO_3^- efflux pathway in the luminal membrane that has yet to be identified. (3) The luminal membrane must not contain

transporters that would allow HCO_3^- secreted into the lumen to re-enter the cell.

To compare the passive permeability characteristics of the basolateral and luminal membranes of guinea-pig pancreatic duct cells to extracellular CO_2 and HCO_3^-, we have examined the changes in intracellular pH that occur in unstimulated, microperfused, interlobular duct segments when either the basolateral or the luminal membrane is exposed to changes in HCO_3^- or CO_2 concentration.

METHODS

Isolation and culture of interlobular ducts

Female Hartley guinea-pigs (350–450 g) were killed by cervical dislocation, in accordance with national guidelines. As described previously (Ishiguro *et al.* 1996*a*), the pancreas was removed and interlobular ducts (diameter 100–150 μm, length 800–1200 μm) were isolated and cultured overnight, during which time the ends of the duct segments sealed spontaneously.

Solutions

The standard Hepes-buffered solution contained (mM): 140 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 D-glucose and 10 Hepes; and was equilibrated with 100% O_2. The Na^+-free Hepes-buffered solution contained *N*-methyl-D-glucamine ($NMDG^+$) in place of Na^+. The standard HCO_3^--buffered solution contained (mM): 115 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 D-glucose and 25 $NaHCO_3$; and was equilibrated with 95% O_2–5% CO_2. The 25 mM HCO_3^-–0% CO_2 solution was gassed with 100% O_2. High-HCO_3^- solutions contained 125 or 145 mM $NaHCO_3$ and the concentration of NaCl was reduced accordingly to maintain osmolarity. The Cl^- concentrations of these solutions were 24 and 4 mM, respectively, compared with 124 mM in the standard HCO_3^--buffered solution. The Na^+-free HCO_3^--buffered solution contained NMDG-Cl in place of NaCl, choline bicarbonate in place of $NaHCO_3$ and 10 μM atropine to avoid any muscarinic effects resulting from the high concentration of choline. Cl^--free solutions were made by replacing Cl^- with glucuronate. In solutions containing NH_4^+, the concentration of Na^+ was reduced to maintain osmolarity. All solutions, except for the high-HCO_3^- solutions and the 25 mM HCO_3^-–0% CO_2 solution, were adjusted to pH 7·4 at 37 °C. The pH values of the high-HCO_3^- solutions equilibrated with 5% CO_2 were in the range 8·2–8·3.

Microperfusion of the isolated ducts

The lumen of the interlobular duct segment was microperfused as described previously (Ishiguro *et al.* 1999). Both ends of the duct were cut open using sharpened needles and one end was cannulated with concentric holding and perfusion pipettes. The duct lumen was perfused at a rate of approximately 10–20 μl min^{-1} while the bath was maintained at 37 °C and continuously perfused at 3 ml min^{-1} in the same direction as the flow of luminal perfusate.

Measurement of intracellular pH (pH_i)

Intracellular pH in the duct cells was estimated by microfluorometry as described previously (Ishiguro *et al.* 1996*a*) using the pH-sensitive fluoroprobe BCECF. After cannulating the duct for luminal perfusion, the duct cells were loaded with BCECF for 10 min by adding the acetoxymethyl ester BCECF-AM (2 μM) to the bathing solution. Perfusion of both bath and lumen was stopped during the 10 min loading period. Experiments were begun after the duct had been perfused with the control solution for 15 min. Small regions of the duct epithelium (10–20 cells) were illuminated alternately at excitation wavelengths of 430 and 480 nm. Values of pH_i were calculated from the fluorescence ratio (F_{480}/F_{430}) measured at 530 nm. The system was calibrated using the high-K^+–nigericin technique (Thomas *et al.* 1979).

Materials

BCECF-AM and dihydro-4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (H_2DIDS) were obtained from Molecular Probes; bafilomycin A_1, forskolin and atropine sulphate from Sigma; and *N*-methyl-*N*-isobutylamiloride (MIA) from Research Biochemicals International.

Statistics

Data are presented as the means ± S.E.M. unless otherwise indicated. Tests for statistically significant differences were made with Student's *t* test for paired or unpaired data.

RESULTS

Effects of CO_2 and HCO_3^- on intracellular pH in unstimulated ducts

To investigate the CO_2 permeability and HCO_3^- transport characteristics of the luminal and basolateral membranes of unstimulated pancreatic duct cells, changes in pH_i were observed following the addition of HCO_3^-/CO_2 first to the luminal perfusate and then to the bath (Fig. 1*A*). Initially both the duct lumen and the bath were perfused with a HCO_3^--free, Hepes-buffered solution. When the luminal perfusate was switched to a HCO_3^--buffered solution containing 25 mM HCO_3^-–5% CO_2, pH_i decreased from 7·25 ± 0·06 ($n = 5$) to 7·04 ± 0·08 over a period of 30 s. The cytosol remained acidified during the following 4 min period of luminal perfusion with the HCO_3^--buffered solution, but when HCO_3^-/CO_2 was removed pH_i quickly returned to its control value.

The sustained acidification observed during exposure of the luminal membrane to HCO_3^-/CO_2 can best be explained by the continuous diffusion of CO_2 from the lumen into the cell. This will occur as a result of the steep P_{CO_2} gradient that is maintained between the lumen and the bath – the P_{CO_2} in the cells attaining a steady-state value somewhere between that of the lumen (equilibrated with 5% CO_2) and the bath (0% CO_2). The diffusion of CO_2 into the cells across the luminal membrane will continue to acidify the cytosol so long as there is an efflux pathway for HCO_3^-, and this most probably occurs via the anion exchangers that are present in the luminal and basolateral membranes (see below). Therefore the steady-state pH that is achieved during this period will reflect a balance between the rate of acidification due to CO_2 entry and HCO_3^- efflux, and the rate of compensatory H^+ extrusion via the Na^+–H^+ exchanger in the basolateral membrane, which is stimulated by the lower pH_i.

In the second part of the experiment, the basolateral membrane was exposed to HCO_3^-/CO_2 by switching the bath perfusate to the HCO_3^--buffered solution. Intracellular pH decreased transiently but then gradually increased to a value which was significantly higher than before HCO_3^-/CO_2 exposure (7·42 ± 0·03 in 3 min, $P < 0·05$). When

HCO_3^-/CO_2 was removed from the perfusate, there was a further transient increase in pH_i followed by a gradual return towards the resting value.

In this case, pH_i recovered quickly from the acidifying effect of CO_2 diffusion into the cells and increased to a higher value. This could be explained either by enhanced H^+ extrusion across the basolateral membrane, which is unlikely given that it did not occur when CO_2 entered across the luminal membrane, or by active HCO_3^- uptake into the cells, necessarily across the basolateral membrane. This would be consistent with our previous data (Ishiguro *et al.* 1996*a*, 1998), which established the presence of a Na^+–HCO_3^- cotransporter in the basolateral membrane.

To distinguish between the separate effects of CO_2 and HCO_3^- on pH_i, a solution was prepared which contained 25 mM HCO_3^- but which was equilibrated with 100% O_2 (pH ~8·2) and thus was nominally free of CO_2. When this solution was applied to the lumen (first open bar, Fig. 1*B*), pH_i decreased by only 0·06 ± 0·01 units ($n = 4$), equivalent to only 26% of the acidification caused by the preceding application of both HCO_3^- and CO_2 (first filled bar). This supports our hypothesis that the initial decrease in pH_i on exposure to luminal HCO_3^-/CO_2 is due to CO_2 entry by diffusion. The small residual decrease could have been due to the presence of a small amount of CO_2 derived from the HCO_3^- in the solution.

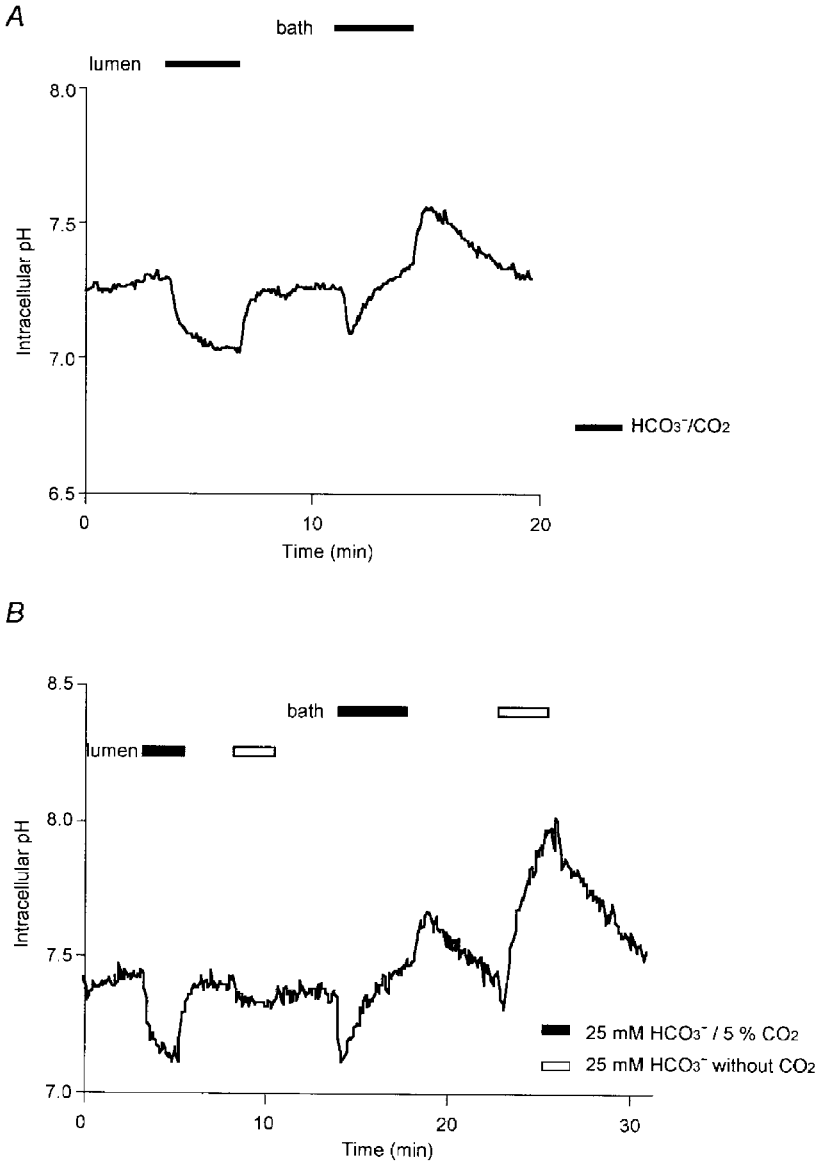

Figure 1. Effects of basolateral or luminal HCO_3^-/CO_2 on intracellular pH

Initially the bath and lumen of guinea-pig pancreatic duct segments were separately perfused with the standard HCO_3^-/CO_2-free Hepes-buffered solution. Thereafter, in *A* (representative of 5 experiments), first the luminal perfusate and then the bath solution was switched to a HCO_3^--buffered solution containing 25 mM HCO_3^-–5% CO_2 (filled bar). In *B* (representative of 4 experiments), the luminal and bath solutions were switched first to 25 mM HCO_3^-–5% CO_2 (filled bar) and then to 25 mM HCO_3^-–0% CO_2 (open bar).

Application of the 25 mM HCO_3^-–0% CO_2 solution to the bath (second open bar) also caused a much reduced transient acidification, but this was followed by a dramatic increase in pH_i to 7·94 ± 0·05 in 3 min. This supports our hypothesis that the secondary increase in pH_i, on exposure of the basolateral membrane to HCO_3^-/CO_2, is due to HCO_3^- entry, exaggerated in these conditions by the absence of the acidifying effect of CO_2 diffusion into the cell.

Dependence of HCO_3^- influx across the basolateral membrane on Na^+ and Cl^-

The transport mechanisms responsible for the influx of HCO_3^- across the basolateral membrane were examined further in ion substitution experiments. When the bath perfusate was switched from the standard Hepes-buffered solution to a Na^+-free HCO_3^--buffered solution (Fig. 2*A*), pH_i decreased rapidly but then remained acidified throughout the 3 min period of Na^+-free perfusion. Restoration of basolateral Na^+ caused pH_i to increase, indicating that the rise in pH_i previously observed during exposure to basolateral HCO_3^-/CO_2 (Fig. 1*A*) was dependent on basolateral Na^+.

When the bath superfusate was switched to a Cl^--free HCO_3^--buffered solution (Fig. 2*B*), pH_i transiently decreased and then increased to 7·93 ± 0·05 in 5 min ($n = 4$). This was a much higher value than that observed in the standard (Cl^--containing) HCO_3^--buffered solution (Fig. 1*A*) and suggests that HCO_3^- influx via the basolateral membrane does not require the presence of basolateral Cl^-. The fact

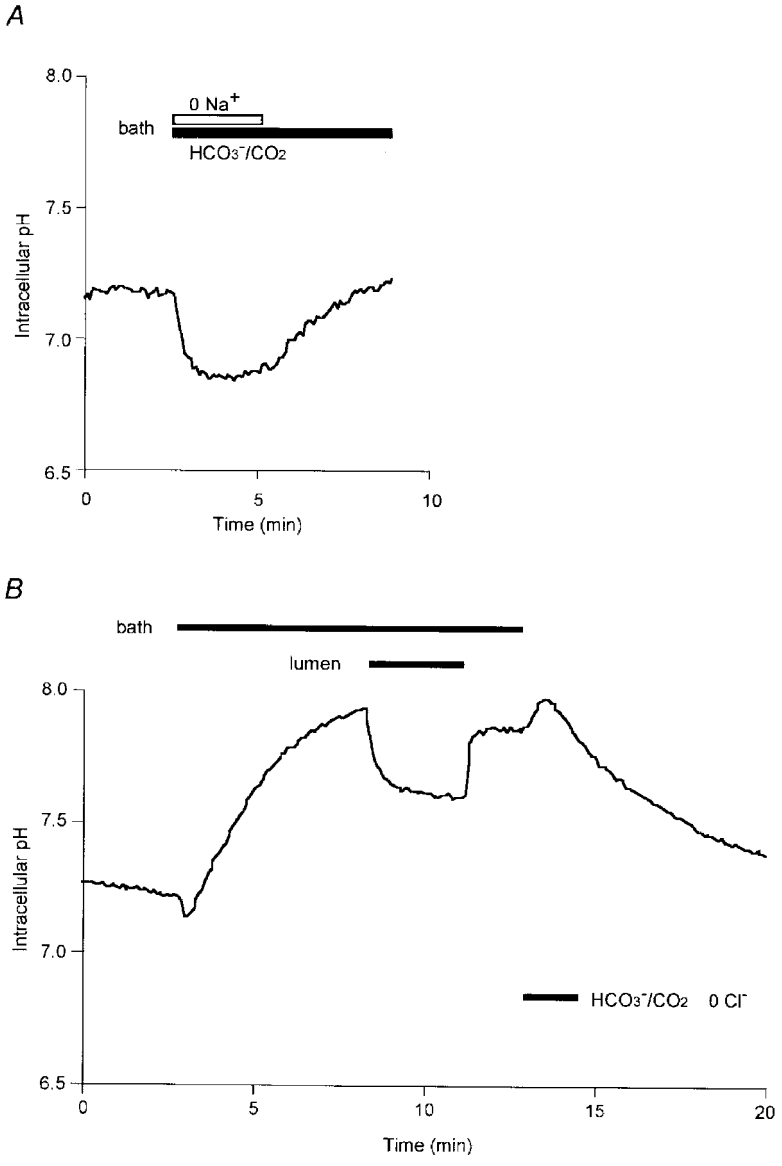

Figure 2. Dependence of HCO_3^- influx across the basolateral membrane on Na^+ or Cl^-

Initially the bath and lumen of the duct segments were separately perfused with the standard Hepes-buffered solution. Thereafter, in *A* (representative of 4 experiments), the bath solution was switched to a Na^+-free HCO_3^--buffered solution, while in *B* (also representative of 4 experiments), the bath solution and then the luminal perfusate were switched to a Cl^--free HCO_3^--buffered solution.

that the rise in pH_i was enhanced by the withdrawal of basolateral Cl^- suggests that a basolateral Cl^-–HCO_3^- exchanger (see below) may have contributed to the uptake of HCO_3^-. Subsequent application of the Cl^--free HCO_3^--buffered solution to the lumen (Fig. 2*B*) caused a decrease in pH_i to a new steady-state value. This would be expected since the rise in cytosolic P_{CO_2} resulting from bilateral exposure to HCO_3^-/CO_2 would inevitably shift pH_i to a lower value.

Effects of higher concentrations of HCO_3^- on intracellular pH

To assess further the ability of the basolateral and luminal membranes to permit the entry of extracellular HCO_3^-, a larger transmembrane gradient of HCO_3^- was applied using a solution containing 125 mM HCO_3^-, 24 mM Cl^-, 5% CO_2. In the experiment shown in Fig. 3*A*, the standard 25 mM HCO_3^- solution and the 125 mM HCO_3^- solution were each applied in turn, first to the luminal membrane and then to the basolateral membrane. Luminal application of the 125 mM HCO_3^- solution (first open bar) caused a rapid decrease in pH_i of 0·20 ± 0·04 ($n = 5$) units, which was not significantly different from the decrease in pH_i observed with the 25 mM HCO_3^- solution (first filled bar; 0·21 ± 0·03). No significant recovery of pH_i was observed during luminal perfusion with the 125 mM HCO_3^- solution, indicating that little if any HCO_3^- entered the cell across the luminal

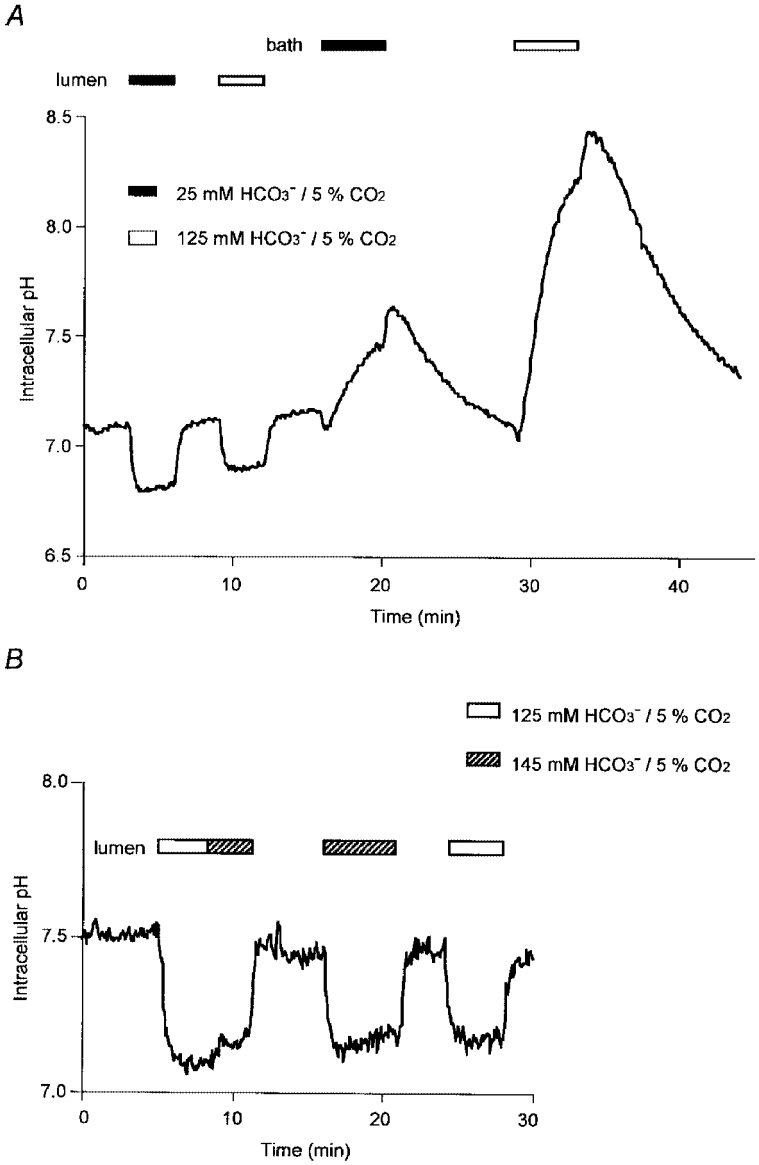

Figure 3. Effects of high extracellular HCO_3^- concentrations on intracellular pH

Initially the bath and lumen of the duct segments were separately perfused with the standard Hepes-buffered solution. Thereafter, in *A* (representative of 5 experiments), the luminal perfusate and then the bath solution was switched first to 25 mM HCO_3^-–5% CO_2 (filled bar) and then to 125 mM HCO_3^-–5% CO_2 (open bar). In *B* (representative of 5 experiments), the lumen was exposed either to 125 mM HCO_3^-–5% CO_2 (open bar) or to 145 mM HCO_3^-–5% CO_2 (hatched bar).

membrane even with a large inward concentration gradient for HCO_3^-. In contrast, when the 125 mM HCO_3^- solution was applied to the basolateral membrane (Fig. 3*A*, second open bar), pH_i increased rapidly to 8·09 ± 0·11. Although this change would have been partly due to HCO_3^- influx via the basolateral Cl^-–HCO_3^- exchanger (driven by the reduction in basolateral Cl^- concentration as in Fig. 2*B*), it indicates a marked difference in the capacity of the luminal and basolateral membranes to transport HCO_3^- into the cells. In particular, it suggests that the activity of the luminal anion exchanger may actually be suppressed under these conditions.

At an even higher luminal HCO_3^- concentration (145 mM) there began to be some evidence of HCO_3^- entry across the luminal membrane (Fig. 3*B*). Initially the 125 mM HCO_3^- solution was applied to the lumen for 3 min (first open bar), during which period no obvious pH_i increase was observed (as previously in Fig. 3*A*). The luminal concentration of HCO_3^- was then raised to 145 mM (first hatched bar) and this caused a slight rise in pH_i over a 3 min period. Direct application of the 145 mM HCO_3^- solution to the lumen (second hatched bar) had a similar effect: pH_i decreased quickly and then increased slowly by 0·10 ± 0·01 units over 5 min ($n = 5$). Direct application of the 125 mM HCO_3^- solution, on the other hand, caused a sustained reduction in pH_i (second open bar). Thus HCO_3^- entry across the luminal membrane appears only to occur at very high luminal HCO_3^- concentrations.

Since the activities of the luminal membrane transporters inevitably change during stimulated secretion, a similar experiment was performed before and during application of 1 μM forskolin, which is known to mimic the effect of secretin in this preparation (Ishiguro *et al.* 1996*b*) by elevating intracellular cyclic AMP (Fig. 4). As before, the lumen was exposed to progressively higher HCO_3^- concentrations at constant P_{CO_2} while the bath remained HCO_3^- free. Application of forskolin itself had little effect on pH_i as reported previously (Ishiguro *et al.* 1996*b*). The pattern of changes in pH_i when the lumen was exposed to the different HCO_3^- concentrations in the presence of forskolin was essentially the same as in the unstimulated state. Thus, whatever changes in transport occur in the luminal membrane during stimulation, there is still little evidence of passive HCO_3^- re-entry into the cells despite the steep concentration gradient.

Recovery of intracellular pH following an acid load

In order to localise the pH regulatory mechanisms responsible for HCO_3^- uptake and H^+ extrusion in unstimulated ducts, we examined whether the recovery of pH_i from an acid load was dependent on basolateral or luminal Na^+. Acid loading was achieved by adding 20 mM NH_4^+ to the bath for 2 min, followed immediately by Na^+ withdrawal from both the bath and lumen. We first examined the recovery of pH_i in ducts in the absence of HCO_3^-/CO_2 (Fig. 5*A*). While the bath and lumen were perfused with the Na^+-free Hepes-buffered solution, there was no recovery of pH_i. Restoration of luminal Na^+ caused no change in pH_i. However, restoration of basolateral Na^+ caused pH_i to return rapidly to the resting value. This indicates that a Na^+-dependent, HCO_3^--independent acid

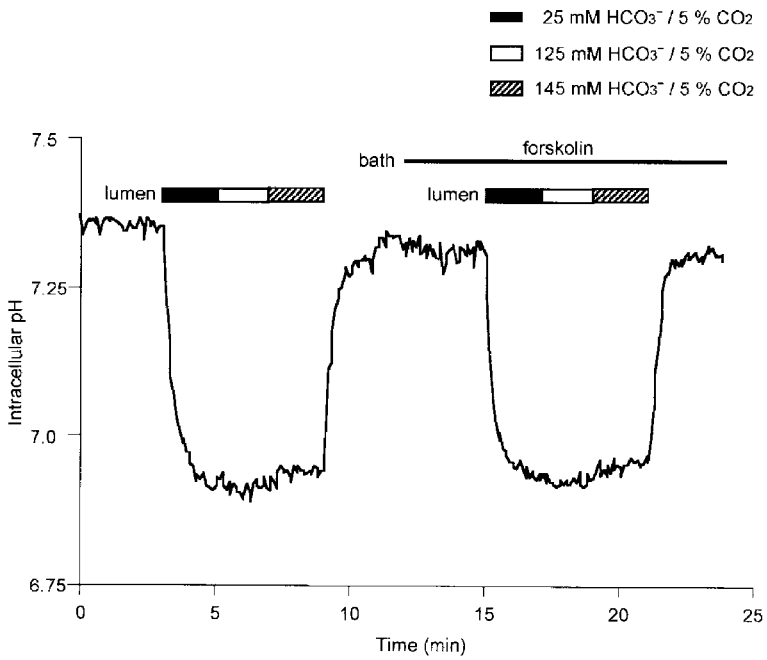

Figure 4. Effect of forskolin stimulation on pH_i changes with high luminal HCO_3^- concentrations
Initially the bath and lumen were separately perfused with the standard Hepes-buffered solution. Thereafter, the lumen was exposed to increasing concentrations of HCO_3^- equilibrated with 5% CO_2. This was then repeated during stimulation with 1 μM forskolin. Data are representative of 4 experiments.

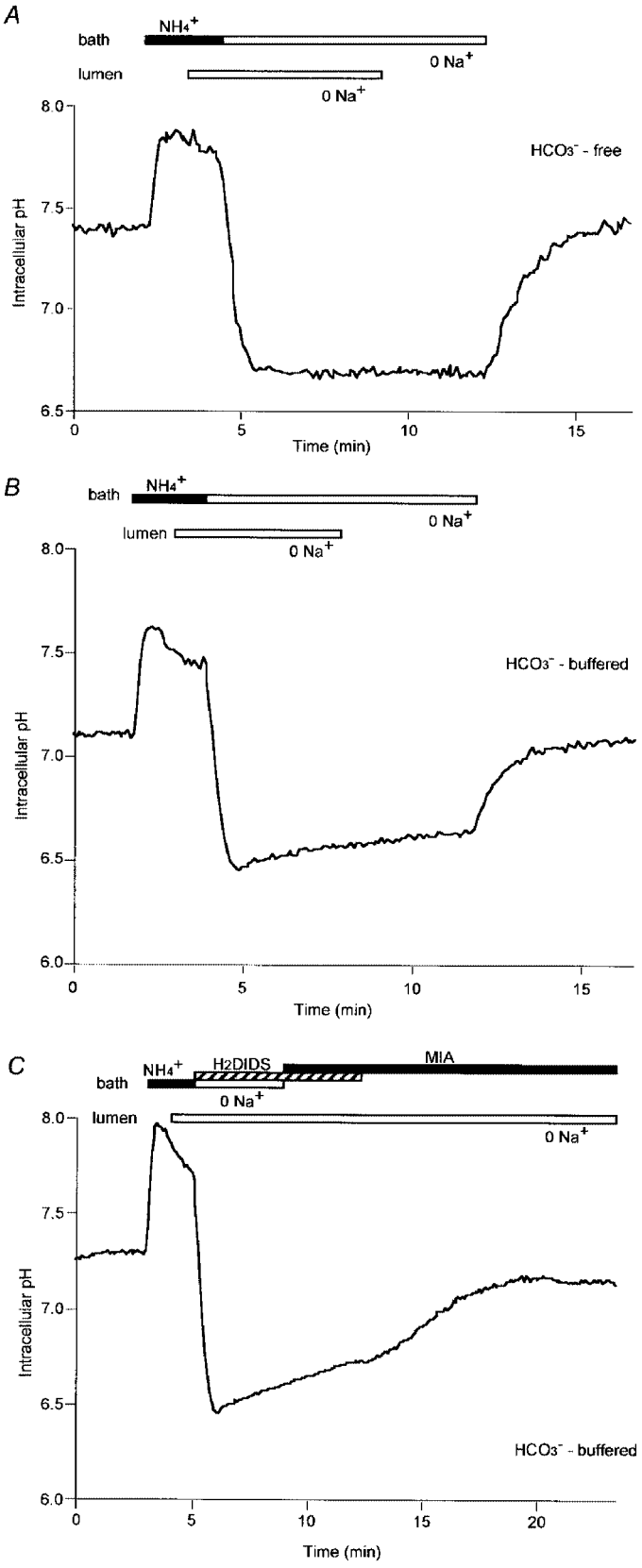

Figure 5. Recovery of intracellular pH from an acid load

Duct cells were acid-loaded by adding 20 mM NH_4^+ to the bath for 2 min followed by Na^+ removal (from the bath and lumen) in the absence (*A*) and presence (*B* and *C*) of HCO_3^-/CO_2. In *C*, H_2DIDS (0·5 mM) and MIA (10 μM) were added to the bath as indicated to block basolateral Na^+–HCO_3^- cotransport and Na^+–H^+ exchange, respectively. Data are representative of at least 4 experiments.

extruder, most probably an Na^+–H^+ exchanger, is located exclusively in the basolateral membrane.

The same experiment was repeated in the bilateral presence of HCO_3^-/CO_2 and rather similar observations were made (Fig. 5*B*). However, in these conditions, a slow but significant recovery of pH_i was observed in the absence of Na^+ from both the lumen and the bath ($0{\cdot}13 \pm 0{\cdot}02$ units in 4 min, $n = 5$). The mechanism responsible for this phenomenon is unclear since it was not inhibited by bafilomycin A_1 (data not shown) and therefore cannot be attributed to the vesicular-type H^+-ATPase previously reported in guinea-pig duct cells (De Ondarza & Hootman, 1997).

While restoration of Na^+ to the lumen did not increase the rate of recovery above this slow basal level, restoration of basolateral Na^+ caused pH_i to return rapidly to its resting value. This suggests that not only the Na^+–H^+ exchanger but also the Na^+–HCO_3^- cotransporter, which we have described previously (Ishiguro *et al.* 1996*a*), are located solely in the basolateral membrane.

To test this hypothesis, the effects of methylisobutyl-amiloride (MIA), an inhibitor of Na^+–H^+ exchange, and the disulphonic stilbene H_2DIDS, an inhibitor of anion transport, were examined using a similar protocol (Fig. 5*C*). In this experiment, the increase in the rate of recovery of pH_i when basolateral Na^+ was restored after acidification (Fig. 5*B*) was blocked by the presence of 10 μM MIA and 0·5 mM H_2DIDS in the bath. When H_2DIDS, which acts as a reversible inhibitor of the basolateral Na^+–HCO_3^- cotransporter, was withdrawn, there was a gradual recovery

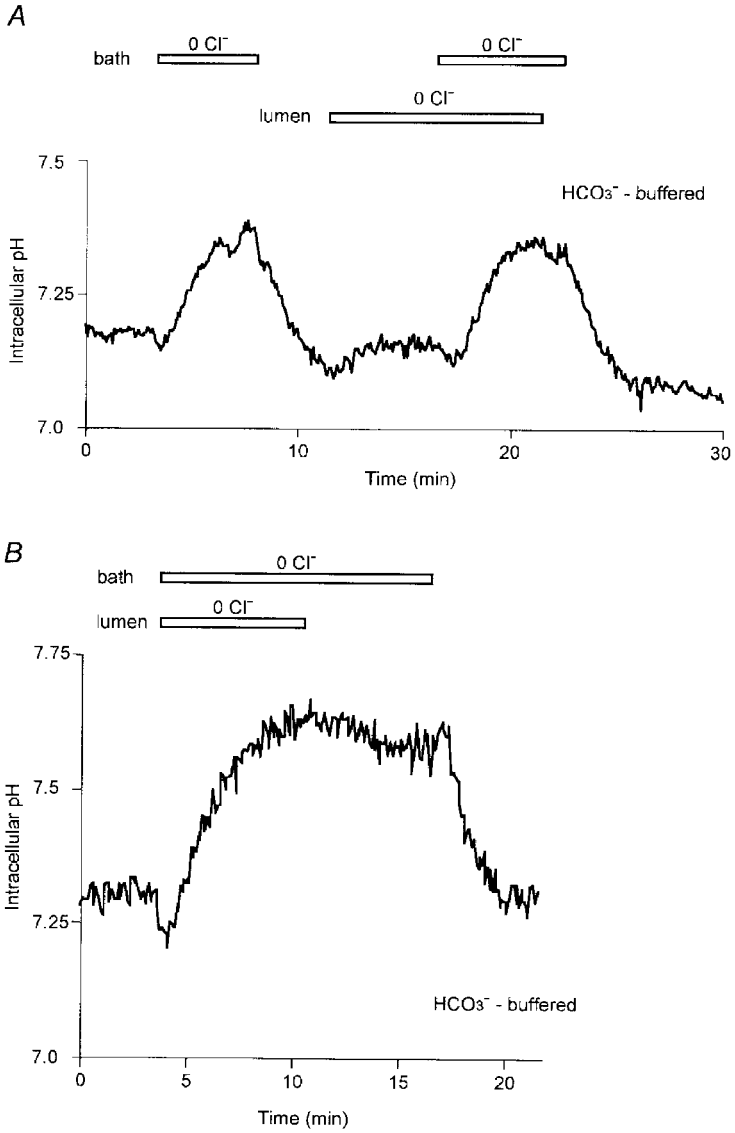

Figure 6. Effects of Cl^- removal on intracellular pH in the presence of HCO_3^-

Initially the bath and lumen of the duct segments were separately perfused with the standard HCO_3^--buffered solution. Thereafter, the luminal perfusate and/or the bath solution was switched to a Cl^--free, HCO_3^--buffered solution (Cl^- replaced with glucuronate) (open bar). Experiments in *A* and *B* are each representative of 5 experiments.

of pH_i in the continued presence of MIA. This is consistent with there being a significant role for HCO_3^- uptake via the cotransporter in the regulation of pH_i.

Effects of Cl^- removal on intracellular pH in the presence of HCO_3^-

Studies on rat pancreatic ducts suggest that HCO_3^- secretion across the luminal membrane may involve the parallel operation of a Cl^-–HCO_3^- exchanger and a cAMP-activated Cl^- channel, the cystic fibrosis transmembrane conductance regulator (CFTR) (Gray *et al.* 1988, 1993; Novak & Greger, 1988). In our work, using guinea-pig pancreatic ducts, we have demonstrated that HCO_3^- transport across the luminal membrane is mediated by Cl^-–HCO_3^- exchange under resting conditions but seems to involve an alternative mechanism during stimulation with secretin (Ishiguro *et al.* 1996*b*, 1998). To determine the localisation of Cl^-–HCO_3^- exchangers in the guinea-pig ducts, we have investigated the effects on pH_i of removing Cl^- from the bath or lumen in the bilateral presence of HCO_3^-/CO_2.

Removal of Cl^- from the bath solution by replacement with glucuronate (Fig. 6*A*) caused a rapid increase in pH_i of $0{\cdot}24 \pm 0{\cdot}03$ units ($n = 5$) over a 4 min period. Removal of Cl^- from the luminal solution caused a slower and much smaller increase in pH_i ($0{\cdot}07 \pm 0{\cdot}01$ units). In Fig. 6*B*, the bath and lumen were initially both switched to the Cl^--free solution and then Cl^- was restored first to the luminal solution and then to the bath. Restoring Cl^- to the lumen caused pH_i to decrease very slowly (by $0{\cdot}06 \pm 0{\cdot}01$ in 6 min, $n = 4$) whereas the subsequent restoration of Cl^- to the bath caused pH_i to decrease much more rapidly (by $0{\cdot}28 \pm 0{\cdot}04$ in 5 min) to the basal value. Taken together the data in Fig. 6 appear to suggest that the activity of the Cl^-–HCO_3^- exchangers in the basolateral membrane of unstimulated pancreatic duct cells is considerably greater than that in the luminal membrane.

DISCUSSION

HCO_3^-/CO_2 permeability of the basolateral and luminal membranes

Transport of HCO_3^- and Cl^- across pancreatic duct epithelium has previously been examined by perfusing the main pancreatic duct of the cat *in vivo* (Case *et al.* 1969; Case & Scratcherd, 1970; Reber *et al.* 1986). These studies revealed that the main duct allowed very little exchange of Cl^- for HCO_3^-, even at the slowest perfusion rates, and therefore must be relatively impermeable to HCO_3^-. Our present paper is the first to study the permeability of small, intra- and interlobular ducts where there is active HCO_3^- secretion (Case & Argent, 1993). By measuring pH_i in microperfused, interlobular duct segments isolated from guinea-pig pancreas, we have demonstrated that (1) both basolateral and luminal membranes are permeable to extracellular CO_2, (2) basolaterally applied HCO_3^- easily gains access to the cell, whereas (3) intraluminally applied HCO_3^- does not. Regardless of the mechanisms involved, this polarity is a basic property of the pancreatic duct epithelium which is necessary to achieve vectorial transport of HCO_3^- from blood to duct lumen.

Only a few previous reports have compared the permeability of the basolateral and luminal membranes of epithelial tissues to HCO_3^-/CO_2. In perfused gastric glands (Waisbren *et al.* 1994), the luminal membranes of both parietal and chief cells were impermeable to HCO_3^- and to CO_2 while the basolateral membranes were permeable to both. Not surprisingly the permeability characteristics of pancreatic duct cells are somewhat different.

Membrane localization of H^+–HCO_3^- transporters

Our present data demonstrate the presence of an Na^+–H^+ exchanger, an Na^+–HCO_3^- cotransporter and an unidentified Na^+-independent acid-extrusion mechanism in the basolateral membrane. Anion exchangers are found in both basolateral and luminal membranes. These results are largely similar to those of a previous study of the rat pancreatic duct (Zhao *et al.* 1994). However, the observation that the Cl^-–HCO_3^- exchanger activity in the basolateral membrane of unstimulated guinea-pig duct cells was greater than that in the luminal membrane was unexpected. We have previously demonstrated that the luminal exchanger plays a role in the spontaneous secretion of HCO_3^- and fluid in guinea-pig ducts (Ishiguro *et al.* 1998). This is achieved by the passive exchange of intracellular HCO_3^- with luminal Cl^- via the anion exchanger in the luminal membrane, while Cl^- ions recycle via a luminal Cl^- conductance. A Cl^-–HCO_3^- exchanger in the basolateral membrane would provide an alternative efflux pathway for intracellular HCO_3^- which would tend to dissipate the accumulation of HCO_3^- across the basolateral membrane whilst favouring the accumulation of Cl^- instead. We would therefore predict that the fluid secreted by unstimulated ducts might be richer in Cl^- than HCO_3^-.

HCO_3^- transport across the basolateral membrane

Two mechanisms have been postulated to account for HCO_3^- accumulation across the basolateral membrane: forward (blood to cell) transport of HCO_3^-, or backward (cell to blood) transport of H^+ derived from carbonic acid (Case & Argent, 1993). The latter concept has been supported by several studies in perfused pancreas preparations, which have shown that a variety of buffer anions can substitute for HCO_3^-.

However, in this study, when solutions containing 25 or 125 mM HCO_3^- were applied to the basolateral membrane, extracellular HCO_3^- entered the cells rapidly. Since this was equally true in the nominal absence of CO_2, these results support the hypothesis that HCO_3^- can be transported actively into the cell. The results are also consistent with a previous study in the isolated perfused cat pancreas in which Ammar *et al.* (1987) altered the pH of the arterial perfusate either at a constant P_{CO_2} or at a constant HCO_3^- concentration. They found that HCO_3^- secretion was independent of arterial pH but critically dependent upon

the arterial HCO_3^- concentration. In our study, the influx of HCO_3^- across the basolateral membrane was largely dependent on basolateral Na^+ and was inhibited by H_2DIDS, and thus is most probably mediated by Na^+–HCO_3^- cotransport as we have suggested previously (Ishiguro *et al.* 1996*a*, 1998).

HCO_3^- transport across the luminal membrane

In the present study we have demonstrated that luminally applied HCO_3^- does not appear to enter the ductal cells until the luminal concentration of HCO_3^- is elevated above 125 mM. Given the presence of an anion exchanger in the luminal membrane this is surprising. When the lumen is perfused with solutions containing 125 mM HCO_3^- and 24 mM Cl^-, the concentration gradients for HCO_3^- and Cl^- across the luminal membrane should strongly favour the exchange of intracellular Cl^- with luminal HCO_3^-, and a consequent rise in pH_i as HCO_3^- enters the cells. The fact that this does not happen suggests that the luminal membrane Cl^-–HCO_3^- exchanger may be inhibited under these conditions, perhaps as a result of the high luminal pH or the high concentration of luminal HCO_3^-. Interestingly, the need for this type of feedback inhibition of the exchanger has already been indicated by computer simulation studies of the rat pancreatic duct (Sohma *et al.* 1996, 1997). Most importantly, this finding again casts doubt on the ability of the exchanger to contribute to net HCO_3^- secretion during secretin stimulation where, in the guinea-pig, the luminal HCO_3^- concentration is already high. Also, as we have previously demonstrated (Ishiguro *et al.* 1998), secretin-stimulated HCO_3^- secretion in the guinea-pig appears to be mediated mainly by a transport mechanism which has, at most, a minimal requirement for Cl^-.

An anion channel with a significant HCO_3^- conductance could account for Cl^--independent HCO_3^- secretion across the luminal membrane. The present finding, that HCO_3^- begins to enter the cells at luminal concentrations above 125 mM, suggests that there may be an anion conductance in the luminal membrane that is permeable to HCO_3^-. Although electrophysiological data are not yet available for the guinea-pig pancreatic duct, a luminal membrane potential of approximately −60 mV would be close to the reversal potential for HCO_3^- at luminal concentrations of this magnitude (assuming that the intracellular HCO_3^- concentration was approximately 10 mM since pH_i was approximately 7·0). Thus, an increase in luminal HCO_3^- concentration from 125 to 145 mM might begin to allow HCO_3^- ions to enter the cells from the lumen and hence cause the small alkalinization observed in Fig. 3*B*. Provided that the membrane potential does not depolarise significantly during stimulation, there could therefore be a small luminally directed electrochemical gradient for HCO_3^- that would drive HCO_3^- secretion via an anion channel.

In summary, we have demonstrated that, while the basolateral membrane of interlobular duct cells from guinea-pig pancreas has powerful mechanisms for HCO_3^- uptake, the luminal membrane presents a significant barrier to the re-entry of secreted HCO_3^- ions from the lumen. Given the likely Cl^- and HCO_3^- gradients across the luminal membrane during stimulated secretion, this can only be explained by a marked inhibition of the luminal anion exchanger. If this is the case, what is the pathway for HCO_3^- secretion across the luminal membrane? There is a possibility that HCO_3^- is secreted via a luminal anion channel. If the membrane potential is large enough, there could be a small electrochemical gradient favouring HCO_3^- efflux to the lumen even when the luminal HCO_3^- concentration exceeds 125 mM. However, if future electrophysiological measurements in the guinea-pig ducts show evidence of significant depolarisation during stimulation, as occurs in the rat (Noval & Pahl, 1993), alternative mechanisms will have to be considered.

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