Independence of apical Cl⁻/HCO₃⁻ exchange and anion conductance in duodenal HCO₃⁻ secretion

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IT HAS LONG BEEN RECOGNIZED that HCO₃⁻-secreting organs such as pancreas, small intestine, bile ducts, and epididymal duct display reduced HCO₃⁻ secretory rates in patients with cystic fibrosis (CF) and are severely affected by the disease (24, 34, 44, 47). Thus a role for a CF-related anion channel in epithelial HCO₃⁻ secretion was envisioned long before the cftr gene was cloned and the CFTR protein expressed and found to be an anion channel (2).

Because of the relatively low HCO₃⁻ conductance of all apical anion channels found in CFTR-expressing cell lines (11, 25, 35), theoretical considerations suggested that the CFTR channel itself or other CFTR-dependent anion channels cannot mediate the high HCO₃⁻ secretory rates found in several gastrointestinal organs. Alternatively, it has been proposed that HCO₃⁻ secretion across the apical membrane in gastrointestinal organs occurs by parallel operation of CFTR Cl⁻/HCO₃⁻ exchangers and Cl⁻/HCO₃⁻ exchangers. In this model, the Cl⁻ channel provides luminal Cl⁻ and acts as a CI⁻ leak pathway to prevent intracellular Cl⁻ accumulation as the exchanger cycles (15, 31, 38, 45). This model gained acceptance because of its plausibility and because a luminal Cl⁻/HCO₃⁻ exchange process has been functionally described in several CFTR-expressing epithelia, such as gallbladder, small and large intestine, and the pancreatic duct system (4, 7, 39).

Because modes to stimulate HCO₃⁻ secretion in CF epithelia could be of considerable advantage for CF patients, the scientific interest in HCO₃⁻ secretion in CF epithelia has strongly increased (6, 37, 43, 45). Several years ago, we observed in isolated rat duodenal mucosa that the HCO₃⁻ secretory response to guanylin was unaltered in the absence of luminal Cl⁻ (13). Further experiments in isolated mouse intestinal mucosa revealed that cAMP, cGMP, and Ca²⁺-dependent agonists lost their HCO₃⁻ secretory potential in the absence of CFTR expression but that apical anion exchange activity was unaltered (42). Thus CFTR channel activation was essential for all forms of agonist-stimulated HCO₃⁻ secretion, but the role of an apical anion exchanger was less evident.

The present study was undertaken to test the basic concept of a coupling between a decrease in intracellular Cl⁻ concentration and stimulation of apical Cl⁻/HCO₃⁻ exchange in the small intestinal epithelium and to investigate whether this mechanism is indeed involved in agonist-stimulated duodenal HCO₃⁻ secretion in rat, a relatively low HCO₃⁻ secretor, or rabbit, a high HCO₃⁻ secretor (possibly because of the contribution by a Cl⁻/HCO₃⁻ exchanger).
Brunner’s glands). We also tested whether the CFTR anion channel itself is the likely transport pathway for agonist-stimulated HCO$_3$ secretion.

**MATERIALS AND METHODS**

**Chemicals and Solutions**

H$^{36}$Cl and $^{22}$NaCl were obtained from Amersham (Braunschweig, Germany); scintillation cocktail was from Packard (Frankfurt am Main, Germany); Rompun was from Bayer, and Ketanest was from Parke-Davis. Unless otherwise specified, all other reagents were purchased from Sigma-Aldrich (Deisenhofen, Germany) (including agarose type III high EEO, DIDS, forskolin, indomethacin, TTX, 8-bromo-cAMP (8-Br-cAMP), and ouabain], Fluka (Deisenhofen, Germany) [including agarose type III high EEO, DIDS, bumetanide, forskolin, indomethacin, TTX, 8-bromo-cAMP (8-Br-cAMP), and ouabain], Fluka (Deisenhofen, Germany) [including agarose type III high EEO, DIDS, bumetanide, forskolin, indomethacin, TTX, 8-bromo-cAMP (8-Br-cAMP), and ouabain].

The luminal solution contained 154 mmol/l NaCl, gassed with 100% O$_2$ and the nutrient solution contained (in mmol/l) 8-bromo-cAMP (8-Br-cAMP), and ouabain], Fluka (Deisenhofen, Germany) [including agarose type III high EEO, DIDS, bumetanide, forskolin, indomethacin, TTX, 8-bromo-cAMP (8-Br-cAMP), and ouabain].

The tissue culture grade, molecular biology grade, or the highest grade available.

**Experimental Methods**

**Animals.** Male New Zealand White rabbits weighing 2,500–3,000 g and female Wistar rats weighing 280–350 g were maintained under standard temperature (21 ± 1°C) and light conditions (12:12-h light-dark cycle). Animals had access to tap water and pelleted food ad libitum.

**Duodenal isolation.** For rat duodenal isolation, the procedure has been described (13). Rabbits were preanesthetized by an intramuscular injection of 100 mg/kg ketamine, 10 mg/kg dihydroxyalcohol, and 0.2 mg/kg atrovent. The abdomen was opened, a 3-cm-long segment of the duodenum was excised, and the animals were killed with an overdose of phenobarbital. The proximal part of the duodenum was excised, and the animals were killed with an overdose of phenobarbital. The proximal part of the duodenum was excised, and the animals were killed with an overdose of phenobarbital.

**Electrophysiology.** The open-circuit transepithelial electrical potential difference (PD) was recorded (DVC-1000 dual voltage clamp; World Precision Instruments, Sarasota, FL) via agar 3 mmol/l KCl bridges. Before the tissue was placed into the chamber, the series resistances of the solutions etc. were assessed, and a fluid resistance compensation was performed before each experiment. The direct-current electrical resistance was determined from the change in PD after sending a current of ~40 μA/cm$^2$ through the mucosa in either direction in a 200-ms interval. The open-circuit condition was chosen because to measure HCO$_3$ secretion, no CO$_2$ or HCO$_3$ may be in the luminal solution. This results in the presence of a concentration gradient, which should not be present under voltage-clamp conditions. In addition, the open-circuit condition is closer to the physiological situation. Under open-circuit conditions, short-circuit current ($I_{sc}$) is calculated from PD and R, and is, of course, not a true short-circuit current, because the transepithelial PD is a driving force for passive ion movement. To assess whether changes in "calculated $I_{sc}$" can be used as an approximate measurement for changes in true $I_{sc}$, we applied voltage-clamp conditions to a complete set of experiments (including flux rates) for the control conditions. In all experiments, a positive PD or a positive $I_{sc}$ reflects a net anion movement from the serosal to the mucosal side. Because we often compare HCO$_3$ secretion with changes in $I_{sc}$, anion movement into the luminal bath is defined as positive in both cases.

**In vitro determination of duodenal HCO$_3$ secretion.** Luminal pH was maintained at 7.4 by a continuous pH stat titration method (Radiometer, Copenhagen, Denmark), and the rate of alkalinization was calculated from the consumption volume of the HCl- or H$_2$SO$_4$-containing titrant solution and is given as micromoles per hour per square centimeter.

**Isotope flux studies.** $^{36}$Cl$^-$, $^{22}$Na$^+$, and $^{3}$H$^-$mannitol flux studies were performed in the open-circuit mode and during voltage clamp to 0 PD. 74 kBq/ml H$^{36}$Cl for $^{22}$Na$^+$ and 62 kBq/ml for $^{3}$H$^-$mannitol (2 mmol/l), respectively, were added either to the serosal or the mucosal solution after it had reached stable HCO$_3$ flux ($J_{HCO_3}$) and electrical parameters.

For each isotope, neighboring pieces of rabbit duodenum and identical sections of age-, weight-, and sex-matched inbred rats were used. After a 30-min period of equilibration, aliquots were taken in 20-min intervals for rat and 15-min intervals for rabbit, radioactivity was determined in a liquid scintillation counter, and the bidirectional flux rates for the respective substance was calculated. The values for $I_{sc}$ and $J_{HCO_3}$ represent the average values of the 20- and 15-min periods, respectively.

**RNA isolation and semiquantitative RT-PCR.** The RNA isolation method, the semiquantitative RT-PCR procedure, and the sequence information of histone 3.3a and 18s rRNA were described in detail previously (1, 41). Homologous primers for rabbit (forward: 5′-ACACATCATCATCACATCATCCATCCATCCATC-3′; reverse: 5′-CATTGGCTCTTATCCTGTGTTC-3′) and rat (forward: 5′-CTGAATCTCAAGTCTAGTGTC-3′; reverse: 5′-CCACTTCACAGAAAAACCC-3′) CFTR were deduced from published sequence information (Genbank accession nos. AF189720 and M89906). The obtained PCR fragments displayed the expected size (696 bp for rabbit and 717 bp for rat CFTR). The identity of the amplifiers was confirmed by restriction analysis.

**Statistics**

All results are expressed as means ± SE; n is the number of separate experiments. Error bars are not shown when included within the symbol. All examinations were performed at least in triplicate. If applicable, P values were determined using the Student’s t-test.

**RESULTS**

HCO$_3$ Secretion, $I_{sc}$, and Bilateral Cl$^-$, Na$^+$, and $^{3}$H-Mannitol Fluxes Under Basal and cAMP-Stimulated Conditions

After stable tissue parameters were reached, basal HCO$_3$ secretion was 1.02 ± 0.2 and mean $I_{sc}$ was 1.65 ±
found in these experiments is well within the values for the CFTR anion conductance in patch-clamp experiments (11, 35).

If an apical Cl-/HCO₃⁻ exchanger was the transporter for cAMP-induced HCO₃⁻ secretion, it should be stimulated during cAMP-stimulated anion secretion. We therefore measured bilateral flux rates for Cl⁻ and Na⁺ under open-circuit conditions (Fig. 2B) and searched for an increase in mucosal-to-serosal Cl⁻ flux. However, we found a marked decrease in the mucosal-to-serosal Cl⁻ flux rate after stimulation with a cAMP analog in the rat duodenum and a very small decrease in rabbit duodenum (Fig. 2B). In both species, serosal-to-mucosal Cl⁻ fluxes strongly increased. The Cl⁻ movements were paralleled by Na⁺ movements, albeit not in a one-to-one fashion. This is most likely due to the open-circuit conditions, with a lumen-negative PD and the unequal ion concentrations in the luminal and serosal perfusate as additional driving forces for ion movement. The decrease in mucosal-to-serosal Cl⁻ and Na⁺ flux is most likely due to the known inhibitory effect of cAMP on electroneutral Na⁺/Cl⁻ absorption mediated by Na⁺/H⁺ exchanger NHE3 and an anion exchanger whose molecular identity in the duodenum is under debate. These data demonstrate that under our experimental conditions, apical anion exchange is inhibited (in rat) or unchanged (in rabbit) rather than stimulated during cAMP-mediated electrogenic anion secretion in rat and rabbit duodenum.

3H⁻ mannitol flux experiments were performed to assess changes in paracellular flux under conditions of the experiments. A net mucosal-to-serosal flux was found for mannitol, and 8-Br-cAMP elicited no significant changes (data not shown). Thus cAMP-induced changes in paracellular flux cannot explain the observed changes in anion secretory rate.

We then performed the same experiments under short-circuit conditions and bilateral identical CO₂/HCO₃⁻-containing solutions in rabbit duodenum (n = 4). Under these circumstances, HCO₃⁻ secretion cannot be measured. The mean \(I_{sc}\) was 0.15 ± 0.18 under resting-state conditions (compared with the calculated \(I_{sc}\) of 1.08 ± 0.16 during open-circuit conditions), and the \(\Delta I_{sc}\) after 8-Br-cAMP was 2.55 ± 0.16 (compared with 2.12 ± 0.18), which decreased to 0.25 ± 0.15 (compared with −0.9 ± 0.22) 1 h after ouabain application. For Na⁺ and Cl⁻, the mucosal-to-serosal flux rates were slightly lower than during open-circuit conditions (possibly due to lack of concentration gradient) and did not significantly change after 8-Br-cAMP application (data not shown), the serosal-to-mucosal Cl⁻ flux was identical under open and short-circuit conditions, and the Cl⁻ secretory response was similar (9.3 ± 3.5 vs. 7.1 ± 2.8). The serosal-to-mucosal Na⁺ flux was lower (16.8 ± 1.3 vs. 21.5 ± 0.5), and 8-Br-cAMP caused a markedly lower rise in serosal-to-mucosal Na⁺ flux (18.1 ± 3.7). The data demonstrate that both the measured \(\Delta I_{sc}\) under short-circuit conditions and the calculated \(I_{sc}\) under open-circuit conditions are relatively close, and both underestimated the CAMP-induced anion secretion. They further demon-
strate that the likely driving force for the strong serosal-to-mucosal Na\(^+\) flux under open-circuit conditions is the strong increase in PD. Third, they show that the Cl\(^-\) secretory response in the presence of 5% CO\(_2~/24\) mM HCO\(_3\)\(^-\) in the luminal bath is identical to if not enhanced from that in its complete absence. These data suggest that HCO\(_3\) concentrations that are likely to occur physiologically within the intestinal lumen do not compromise the Cl\(^-\) secretory response [contrasting the recent report of a strong inhibition of the CFTR Cl\(^-\) conductance by external HCO\(_3\) in a cultured cell line (32)].
Ion Substitution Experiments

If cAMP-stimulated duodenal HCO₃⁻ secretion is primarily mediated by an apical Cl⁻/HCO₃⁻ exchange process, removal of Cl⁻ should strongly inhibit this process. Therefore, we first studied the effect of complete Cl⁻ removal from the system. In bilateral Cl⁻-free conditions, basal HCO₃⁻ secretion decreased in both species to the same values as found in the absence of luminal Cl⁻, and 8-Br-cAMP elicited a HCO₃⁻ secretory response that was markedly higher than in the presence of Cl⁻ (Fig. 3). This rules out an essential role of Cl⁻/HCO₃⁻ exchange in cAMP-stimulated duodenal HCO₃⁻ secretion.

It could be argued that the CFTR channel is permeable for HCO₃⁻ in the absence but not in the presence of intracellular Cl⁻. Therefore, we next tested the effect of selective removal of luminal Cl⁻. The luminal substitution of Cl⁻ by the gluconate ion increased Iᵯ in both epithelia but reduced basolateral ouabain-sensitive (as determined in the experiments of Fig. 1) HCO₃⁻ secretory rate by ~40% in rat (Fig. 4A) and rabbit duodenum (Fig. 4B), suggesting that a substantial part of basal HCO₃⁻ secretion was mediated by luminal Cl⁻/HCO₃⁻ exchange. However, 8-Br-cAMP led to an increase of HCO₃⁻ secretion that was even somewhat higher (~20% in both epithelia) than after stimulation in the presence of luminal Cl⁻. In the absence of luminal Cl⁻, ouabain does not inhibit Iᵯ. This could be either due to the fact that in the absence of luminal Cl⁻, a negative membrane potential (which would be generated by basolateral K⁺ channel and Na⁺-K⁺-ATPase activity) is not required for Cl⁻ flux via apical Cl⁻ channels.

Because Cl⁻ is transported across the epithelium during luminal Cl⁻-free conditions, and this Cl⁻ could potentially be exchanged for intracellular HCO₃⁻ via apical Cl⁻/HCO₃⁻ exchange, we measured the actual amount of Cl⁻ that is transported or leaks across the mucosa into the luminal compartment. The rate of serosal-to-mucosal Cl⁻ movement in the absence of luminal Cl⁻ was 10.3 μmol cm⁻² h⁻¹; thus the Cl⁻ concentration in the luminal bulk solution was ~2 μM at the time of 8-Br-cAMP addition. This value is far lower than any Kᵦ value for external Cl⁻ reported for any apical anion exchange process within the intestine or for any cloned and expressed anion exchanger protein studies so far. This makes it unlikely that an apical Cl⁻/HCO₃⁻ is not inhibited by luminal Cl⁻ substitution.

Effect of Bumetanide in the Presence and Absence of Luminal Cl⁻

Bumetanide inhibits the basolateral Na⁺-K⁺-2Cl⁻ cotransporter, which could lead to a decrease in intracellular Cl⁻ concentration ([Cl⁻]), and an increase in Cl⁻ import via the apical Cl⁻/HCO₃⁻ exchanger, in

![Fig. 2. Serosal-to-mucosal (s > m; A and C) and mucosal-to-serosal (m > s; B and D) Na⁺ and Cl⁻ fluxes, averaged for 20 min in rat (A and B) and 15 min in rabbit (C and D) before and after stimulation with 8-Br-cAMP. It is clear that 8-Br-cAMP increases JₑHCO₃⁻ and Iᵯ as well as serosal-to-mucosal Na⁺ and Cl⁻ fluxes while inhibiting mucosal-to-serosal Na⁺ and Cl⁻ flux strongly in rat (A and B); all changes are significant at P < 0.05 or 0.01; n = 6 for rat and 5 for rabbit) and weakly in rabbit duodenum (C and D; not significant). Mucosal-to-serosal Cl⁻ flux is to a large extent DIDS sensitive and therefore likely mediated by apical Cl⁻/HCO₃⁻ exchange, and its inhibition is consistent with the current model of cAMP inhibition of coupled Na⁺/H⁺ and Cl⁻/HCO₃⁻ exchange. We found no stimulation of apical Cl⁻/HCO₃⁻ exchange during cAMP-mediated anion secretion, as would have to be expected if a coupling of these 2 pathways was mandatory for HCO₃⁻ secretion. B and F: 8-Br-cAMP-induced increase in serosal-to-mucosal flux of Cl⁻ and Na⁺ as well as JₑHCO₃⁻ and Iᵯ, all averaged over the first 15 min after stimulation, in rat (E) and rabbit (F) duodenum. Interestingly, the increase in serosal-to-mucosal Cl⁻ flux by the cAMP analog (significant for P < 0.05) was relatively similar in rat and rabbit duodenum, despite a very different ΔJₑHCO₃⁻/ΔJₑCl⁻ ratio. The ratio of change in Cl⁻ flux to ΔJₑHCO₃⁻ was 11:1 in rat and 5:1 in rabbit anion secretory response.](http://ajpgi.physiology.org)}
inhibition of basolateral Cl\(^{-}\) uptake, is inhibited by luminal Cl\(^{-}\) substitution. This demonstrates that luminal Cl\(^{-}\) substitution is indeed effectively inhibiting apical Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchange.

**DIDS and 5-nitro-2-(3-phenylpropylamino)-benzoate Experiments**

The brush-border membrane Cl\(^{-}\)/HCO\(_3\) exchanger in rat and rabbit duodenum is inhibited by the stilbene derivative DIDS. The luminal application of DIDS in a concentration of 1 mmol/l approximately halved basal ouabain-sensitive HCO\(_3\) secretion in rat and rabbit; but again, the 8-Br-cAMP-induced HCO\(_3\) secretory response was slightly higher than in the absence of luminal DIDS (Fig. 6A for rabbit duodenum only, but

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**Fig. 4.** \(J_{\text{HCO}_3}\) (right axis) and \(I_{\text{sc}}\) (left axis) in rat (A) and rabbit (B) isolated duodenum before and after removal of Cl\(^{-}\) from the luminal perfusate and subsequent stimulation with 8-Br-cAMP. Luminal Cl\(^{-}\) removal resulted in a significant reduction of the basal HCO\(_3\) secretory rate (\(P < 0.05; n = 5\)), suggesting that a substantial part of basal HCO\(_3\) secretion is mediated by apical Cl\(^{-}\)/HCO\(_3\) exchange. The secretory response to 8-Br-cAMP was even somewhat larger than in the control (not significant for \(n = 5\)).

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**Fig. 5.** \(J_{\text{HCO}_3}\) (right axis) and \(I_{\text{sc}}\) (left axis) in rat isolated duodenum before (A) and after (B) removal of Cl\(^{-}\) from the luminal perfusate and subsequent application of bumetanide (10\(^{-4}\) M) followed by stimulation with 8-Br-cAMP. A: as expected of an agent that blocks Na\(^{+}\)/K\(^{+}\)-2Cl\(^{-}\) cotransport, bumetanide application resulted in a decrease in basal \(I_{\text{sc}}\) and a reduced \(I_{\text{sc}}\) response. HCO\(_3\) secretion, however, increased after bumetanide application, both in the basal state and when applied during the plateau phase after 8-Br-cAMP addition (data not shown). This suggested that an inhibition of basolateral Cl\(^{-}\) influx stimulates apical Cl\(^{-}\) uptake via Cl\(^{-}\)/HCO\(_3\) exchange. B: in the absence of Cl\(^{-}\) in the luminal bath, no increase in HCO\(_3\) secretion was observed after bumetanide application, suggesting that luminal Cl\(^{-}\) removal is effective in reducing Cl\(^{-}\) concentration near the apical membrane of the enterocytes to values low enough to inhibit the apical Cl\(^{-}\)/HCO\(_3\) exchange. 8-Br-cAMP results in a significantly reduced \(I_{\text{sc}}\) (\(P < 0.01\) but normal HCO\(_3\) secretory response (not significant) in both conditions, compared with the stimulation in the absence of bumetanide.
rat yielded qualitatively similar results). To test whether DIDS exerts its effect on basal $\text{HCO}_3^-$ secretion primarily via inhibition of apical anion exchange or via inhibition of a DIDS-sensitive anion conductance or the basolateral Na$^+$/HCO$_3^-$ cotransporter, we then tested the effect of luminal DIDS on basal and stimulated $\text{HCO}_3^-$ secretion in the complete absence of Cl$^-$ in the system (Fig. 6B). In the absence of Cl$^-$, luminal DIDS had only a minimal effect on HCO$_3^-$ secretion (0.3 vs. 2.2 μeq·cm$^{-2}$·h$^{-1}$ in the presence of Cl$^-$; B).

DISCUSSION

This study investigates the involvement of an apical Cl$^-$ /HCO$_3^-$ exchange process in basal and cAMP-stimulated duodenal HCO$_3^-$ secretion. It was found that although a Cl$^-$ /HCO$_3^-$ exchanger mediates an important part of the basal HCO$_3^-$ secretory rate, its activity is neither necessary for, nor stimulated during, cAMP-induced HCO$_3^-$ secretion. This finding was observed in rats, a species with rather low pancreatic and duodenal HCO$_3^-$ secretory rates, and rabbits, one with high rates comparable with humans. In both species, a DIDS-insensitive, NPPB-sensitive, cAMP-activated anion conductance mediates a secretory response with a strong predominance of Cl$^-$ over HCO$_3^-$ in the secreted anions.

Gastrointestinal and hepatobiliary HCO$_3^-$ secretion has been recognized as a crucial transport process for the maintenance of mucosal integrity, enzymatic digestion, and Cl$^-$ absorption (8, 9, 29, 31). All HCO$_3^-$-secreting epithelia in CF patients show abnormal function, likely related to pH changes of their secretions.
Recent evidence suggests that CFTR mutations with a strong HCO\textsubscript{3}\,-secretory defect are associated with severe pancreatic and intestinal disease (6). Thus the question of whether the transport of HCO\textsubscript{3}\,- occurs via the CFTR protein itself or via a CFTR-regulated anion transporter may have considerable clinical implications. In the first instance, no option exists to restore HCO\textsubscript{3}\,- secretory function other than a repair of the defect in the CFTR protein itself. If another protein is responsible for HCO\textsubscript{3}\,- transport, then the potential exists to activate this transport process independently of CFTR activation.

A number of theoretical considerations has suggested that the high agonist-induced HCO\textsubscript{3}\,- secretory rates found in duodenal or pancreatic epithelium cannot be transported by the CFTR protein. Apical anion channels in epithelial cell lines with properties of the CFTR anion conductance, as well as CFTR-like channels in CFTR expression systems, had HCO\textsubscript{3}\,- to Cl\,- permeability ratios between 1:3 and 1:8 (11, 16, 35). By extrapolating intracellular anion concentrations and membrane potentials that had been measured in primitive epithelial cell lines to the conditions in native epithelia, it was calculated that under "physiological" driving forces these apical anion conductances would secrete Cl\,- rather than HCO\textsubscript{3}\,- (12, 25). The actual intestinal and pancreatic HCO\textsubscript{3}\,- transport pathways were therefore thought to be CFTR-dependent anion channels with a higher conductivity and HCO\textsubscript{3}\,- permeability than CFTR channels or Cl\,-/HCO\textsubscript{3}\,- exchangers coupled to CFTR channel activity via the intra- or extracellular Cl\,- concentration. But these hypotheses were neither substantiated nor ruled out.

The most attractive hypothesis is a coupling between an apical anion exchanger and the CFTR anion channel. Both DRA, a recently identified intestinal anion transport protein whose mutational defects are the molecular basis for the Finnish familial chloride diarrhea, and putative anion transporter (PAT)1, a recently identified anion transport protein from the same gene family, are apically expressed in gastrointestinal and pancreatic epithelia (19, 46), and recent studies suggest both structural (27, 28) and functional interaction of CFTR and DRA/PAT1 (23).

Effective removal of Cl\,- from the lumen inhibits any apical Cl\,-/HCO\textsubscript{3}\,- exchange process, and the stilbene DIDS has been shown to inhibit DRA and PAT1. Both Cl\,- removal from and the addition of DIDS to the luminal perfusate inhibited a substantial part of the ouabain-sensitive basal HCO\textsubscript{3}\,- secretion despite having completely different effects on PD and R. This suggests that an apical Cl\,-/HCO\textsubscript{3}\,- exchanger mediates a significant part of basal HCO\textsubscript{3}\,- secretion in the duodenum of rat and rabbit and makes it unlikely that the observed changes in basal HCO\textsubscript{3}\,- secretion were secondary to changes in paracellular HCO\textsubscript{3}\,- movement. The application of luminal DIDS has a minimal effect on the residual HCO\textsubscript{3}\,- secretion after complete removal of Cl\,-, demonstrating that indeed the same HCO\textsubscript{3}\,- transport pathway is inhibited by these maneuvers, namely, an apical anion exchange process. It rules out

Fig. 7. Semiquantitative RT-PCR analysis of CFTR mRNA expression levels in different segments of the gastrointestinal tract in rat (B) and rabbit (C) intestine. Exemplary for all PCR experiments, Fig. 7A shows similar amplification efficiency of the gene of interest (CFTR) and the control gene (histone 3.3a) from rabbit duodenal mucosa. The bars depict relative expression levels of rat CFTR vs. 18S RNA (B) and rabbit CFTR vs. histone 3.3a (C) (n = 3). In rabbit, the expression levels are also given (as ODI ratios) in stomach and kidney, organs that are also known to express CFTR. By comparison, duodenal CFTR expression is high.
a substantial contribution of the highly DIDS-sensitive outwardly rectifying Cl− channel (ORCC) (10), a strong inhibition of the basolateral Na+−HCO3 cotransporter by luminal DIDS application, or a major effect of external DIDS on the HCO3− conductivity of the CFTR channel. In contrast, the cAMP-induced HCO3− secretory response was unaffected by Cl− removal or luminal DIDS. The strong HCO3− secretory response in the complete absence of Cl− was also seen in the duodenum of CFTR +/+ but not of −/− mice (I. Blumenstein, unpublished data). The absence of HCO3− in the luminal solution also makes an electrogenic 1 HCO3−-to-2 HCO3− exchange unlikely (23). Together, the data argue against the concept that Cl−/HCO3− exchange is involved in cAMP-dependent duodenal HCO3− secretion.

A lack of effect of luminal Cl− removal on intraluminal alkalization has been observed in isolated pancreatic and epididymal ducts (5, 17). However, it has been argued that movement of serosal Cl− through the epithelium may cause Cl− concentrations near the brush-border membrane sufficiently high to allow uninhibited operation of an apical anion exchanger. We therefore sought for a way to stimulate the apical anion exchanger. In rat duodenum, bumetanide inhibits basolateral Na+/K+/2Cl− cotransport and reduces Isc but stimulates HCO3− secretion. This stimulation is completely dependent on the presence of Cl− in the luminal bath, whereas subsequent stimulation by cAMP analogs is unaffected. The likely explanation is that the inhibition of basolateral Na+/K+/2Cl− cotransport results in a decrease in [Cl−]i, which in turn stimulates apical Cl− uptake by Cl−/HCO3− exchange. Thus the basic principle of apical Cl−/HCO3− exchange activation by low [Cl−]i is applicable. Cl− removal from the luminal bath appears to effectively inhibit apical Cl−/HCO3− exchange.

What then is the likely mechanism for agonist-stimulated duodenal HCO3− secretion? Its complete absence in CFTR −/− mice is indicative of a CFTR-dependent mechanism (42). The involvement of ORCCs seems improbable because of their DIDS sensitivity (10, 21, 22). Purinergic, receptor-activated, Ca2+/CaM-dependent channels of the CaCC family are inhibited by CFTR activation (26, 48). Cl− channel CIC-2 expression has also been found to be apically located in the intestine of normal and CFTR −/− mice (20), but these are acti-
vated by osmotic swelling and hyperpolarization (36, 40, 49), the opposite of which occurs during cAMP-dependent stimulation of enterocytes. Moreover, recently a tight-junctional localization of CIC-2 has been described in small intestine (14). Very recently, a CIC-4 channel has been colocalized with CFTR in mouse and human intestine (30), but again, its properties make it unlikely that this channel mediates the cAMP-stimulated HCO\textsubscript{3} secretory current.

Thus none of the anion transport mechanisms discussed above appears to be a more likely candidate for agonist-stimulated duodenal HCO\textsubscript{3} secretion than the CFTR protein itself. The percentage of HCO\textsubscript{3} to Cl\textsuperscript{−} in the rat and rabbit (as well as mouse; data not shown) duodenal secretory response is fully compatible with any HCO\textsubscript{3}/Cl\textsuperscript{−} permeability ratio measured for CFTR expression systems. NPPB sensitivity is certainly not specific for the CFTR channel, but a lack of NPPB inhibition on I\textsubscript{sc} and HCO\textsubscript{3} secretory response would have been noteworthy; however, this was not found. Duodenal CFTR mRNA expression levels were found to be in the range of those for electroneutral ion exchangers like DRA (19), which, given the high transport capacity of a channel compared with an exchanger, forms the basis for substantial intestinal anion flux via CFTR.

For the measurement of HCO\textsubscript{3} secretion, we studied the tissues in the open-circuit mode. In a leaky tissue like the duodenum, changes in PD could elicit considerable ion fluxes via the tight junctions. However, the marked increase in tissue resistance after the removal of Cl\textsuperscript{−} did not decrease the HCO\textsubscript{3} secretory response to cAMP analogs. Because an increase in PD negativity would inhibit paracellular HCO\textsubscript{3} secretion, the observed increase is likely transcellular.

To better understand the observed increase in cAMP-induced Na\textsuperscript{+} flux under open-circuit conditions, we measured all parameters except HCO\textsubscript{3} secretion under voltage-clamp conditions in rabbit duodenum (in this instance with CO\textsubscript{2}/HCO\textsubscript{3} on both sides of the epithelium). We found a markedly lower cAMP-induced serosal-to-mucosal Na\textsuperscript{+} efflux, demonstrating that the strong cAMP-induced serosal-to-mucosal Na\textsuperscript{+} efflux under open-circuit conditions is indeed due to the stimulation-associated increase in PD negativity and that short circuiting can actually be achieved quite successfully in rabbit duodenum. The Cl\textsuperscript{−} secretory response was not significantly affected. The surprising finding was that the accompanying I\textsubscript{sc} increase still was markedly lower than the Cl\textsuperscript{−} secretory response. We do not understand this difference. Possible explanations are a relatively large conductive pathway for K\textsuperscript{+} in the brush-border membrane or basolateral anion uptake via electrogenic pathways.

We conclude that an apical Cl\textsuperscript{−}/HCO\textsubscript{3} exchange process mediates a substantial part of basal duodenal HCO\textsubscript{3} secretion. Wherever present in the apical membrane, this exchanger will allow the generation of high luminal HCO\textsubscript{3} (or CO\textsubscript{2} concentrations, if coupled with a Na\textsuperscript{+}/H\textsuperscript{+} exchanger) and low luminal Cl\textsuperscript{−} concentrations. The Cl\textsuperscript{−}/HCO\textsubscript{3} exchanger, however, is not the transport pathway for agonist-stimulated HCO\textsubscript{3} secretion. While stimulated by low [Cl\textsuperscript{−}]\textsubscript{i}, it is not stimulated by an activation of electrogenic anion secretion, possibly because basolateral Cl\textsuperscript{−} uptake mechanisms effectively prevent a secretion-associated decrease in [Cl\textsuperscript{−}]\textsubscript{i}.

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