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CHARACTERISTICS OF FLUID SECRETION FROM ISOLATED RAT PANCREATIC DUCTS STIMULATED WITH SECRETIN AND BOMBESIN

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SUMMARY

1. Micropuncture techniques were used to study the cellular mechanisms of fluid secretion by interlobular ducts isolated from the pancreas of copper-deficient rats.

2. Perifusing ducts with a calcium-free buffer containing 5 mM-EGTA reduced the volume of fluid secreted in the presence of 10 nM-bombesin by 62%, whereas fluid secretion measured in the presence of 10 nM-secretin was reduced by only 26%.

3. The anion selectivities of the fluid secretions evoked by secretin and bombesin were different. The anion sequence for secretin was: $Br^- = I^- = NO_3^- = Cl^-$ (1·0) $\gg$ thiocyanate = gluconate (0·3); whereas the sequence for bombesin was: $Br^- = Cl^-$ (1·0) > $I^- = NO_3^-$ (0·6) > thiocyanate = gluconate ($\sim$ 0·3).

4. SITS (4-acetamido-4′-isothiocyanatostilbene-2,2′-disulphonic acid; mM), reduced fluid secretion measured in the presence of bombesin by 61%, but had no effect on the response to secretin.

5. The K^+ channel blockers, barium (3 mM) and tetraethylammonium (TEA; 10 mM), inhibited fluid secretion measured in the presence of both secretin and bombesin by between 52 and 66%.

6. From these results, we conclude that secretin and bombesin may utilize different intracellular signalling pathways and, furthermore, may activate different anion secretory mechanisms within the pancreatic ductal epithelium. However, the effect of the potassium channel blockers is consistent with both peptides activating secretory mechanisms which are electrogenic, and which depend for their operation on potassium efflux across the basolateral membrane of the duct cell.

INTRODUCTION

The pancreatic ductal tree is a network of branching tubules whose primary function is to conduct digestive enzymes (secreted by acinar cells) into the duodenum (for a review see Case & Argent, 1989). However, in addition to acting as a simple conduit, the ductal tree is an ion-transporting epithelium which secretes a

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bicarbonate-rich isotonic fluid. This alkaline secretion flushes digestive enzymes down the ductal tree towards the gut, and is also partly responsible for neutralizing acid chyme which enters the duodenum from the stomach. To date, it has generally been assumed that secretin and vasoactive intestinal peptide are the most important physiological stimulants of duct cell function (Case & Argent, 1989); however, we have recently discovered that the tetradecapeptide bombesin, which had previously been shown to stimulate enzyme secretion from acinar cells (Deschodt-Lanckman, Robberecht, De Neef, Lammens & Christophe, 1976), is also a potent activator of ductal fluid transport in the rat (Ashton, Argent & Green, 1990).

The aim of this study was to compare the characteristics of the fluid secretions evoked by bombesin and secretin from rat pancreatic ducts. Our results suggest that these two peptides utilize different intracellular signalling pathways and, furthermore, that they may activate different anion secretory mechanisms within the ductal epithelium.

METHODS

Animals

Male Wistar rats (125–150 g), obtained from the Manchester University breeding colony, were fed a copper-deficient diet for 6–8 weeks as previously described, but without the addition of copper-chelating agents (Arkle, Lee, Cullen & Argent, 1986). Copper deficiency causes a non-inflammatory atrophy of pancreatic acinar cells but leaves the ducts structurally and functionally intact (for references see Argent, Arkle, Cullen & Green, 1986; Arkle *et al.* 1986). As a starting point for duct isolation this preparation has two advantages. First, the proportion of duct cells in the gland is increased and second, the content of potentially harmful digestive enzymes is markedly reduced.

Isolation and culture of interlobular ducts

Copper-deficient rats were killed by cervical dislocation and the pancreas was removed. Interlobular ducts were then microdissected from the gland and maintained in tissue culture for up to 48 h as previously described (Argent *et al.* 1986; Arkle *et al.* 1986). We have already shown that ducts isolated in this way possess morphological, biochemical and secretory characteristics which are typical of ducts within the intact pancreas of copper-replete rats (Argent *et al.* 1986; Arkle *et al.* 1986).

Micropuncture experiments

Fluid secretion by the cultured ducts was measured using micropuncture techniques as described in previous reports (Argent *et al.* 1986; Ashton *et al.* 1990). Secretion rates are expressed as nl h^{-1} (nl duct epithelium)$^{-1}$, and only one fluid collection was made from each duct.

Solutions

During the micropuncture experiments, the ducts were perifused with a Krebs–Ringer bicarbonate buffer. The standard buffer contained (mmol l^{-1}): NaCl, 120; KCl, 4·5; $CaCl_2$, 2·5; $MgSO_4$, 1; NaH_2PO_4, 1; $NaHCO_3$, 25 and D-glucose, 5. This fluid was heated to 37 °C, and gassed with 5% CO_2, 95% O_2 to set pH at 7·4. In the nitrate, bromide and iodide replacement experiments, all the chloride salts in the standard perifusion buffer were replaced with salts of the appropriate anion. For the thiocyanate and gluconate replacement experiments, all the NaCl and KCl in the standard buffer were replaced by either thiocyanate or gluconate salts, and the $CaCl_2$ was replaced with $CaSO_4$. The osmolality of the standard perifusion fluid, and the five anion replacement solutions, was checked and found to be 281 ± 4 mosmol kg^{-1} ($n = 6$). In some experiments we reduced the $CaCl_2$ concentration in the standard buffer to 0·5 mmol l^{-1}, or used a nominally Ca^{2+}-free solution prepared by omitting all the $CaCl_2$ from the standard buffer and adding ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*′-tetraacetic acid (EGTA; 5 mmol l^{-1}). The

transport inhibitors 4-acetamido-4′-isothiocyantostilbene-2, 2′-disulphonic acid (SITS), tetraethylammonium (TEA) and barium (chloride salt) were all dissolved in the standard perifusion buffer at the final concentrations indicated in the Results.

Bombesin and secretin were made up as concentrated stocks in the appropriate perifusion fluid from which bicarbonate had been omitted, but which contained 5% (w/v) bovine serum albumin. These stock solutions were infused into the tissue bath at a rate calculated to produce a final hormone concentration of 10 nM. Albumin alone, at the final concentration used in these studies, has no effect on ductal fluid transport.

Chemicals

Culture media, serum and glutamine were obtained from Flow Laboratories, insulin (human velosulin 100 units ml^{-1}) from Wellcome and dexamethasone (4 mg ml^{-1}) from Organon Laboratories. Bombesin and bovine serum albumin (fraction V) was obtained from Sigma. Pure natural porcine secretin was purchased from Professor V. Mutt, Karolinska Institutet, Stockholm, Sweden. All other chemicals were of the highest purity available.

Statistical analyses

All values are expressed as the means ± standard error of the mean (n = number of observations). Statistical comparisons were made using analysis of variance (ANOVA) and the Student–Newman–Keuls (SNK) range test. The SNK test allows comparison of several treatments at once, upon the basis of a significant ANOVA, and overcomes the possible errors produced by multiple t testing. Differences were considered significant at the 5% level.

RESULTS

The calcium dependency of ductal fluid secretion

To test whether ductal fluid transport evoked by secretin and bombesin might be activated by different intracellular signals, we examined the sensitivity of these responses to extracellular calcium removal. Figure 1 shows that when ducts were perifused with a nominally Ca^{2+}-free buffer containing 5 mM-EGTA, fluid secretion measured in the presence of secretin was reduced by 26%. On the other hand, when bombesin was the stimulant, fluid secretion was inhibited by 62% with the Ca^{2+}-free buffer, and by 27% when the perifusion fluid contained 0·5 mM-Ca^{2+} (Fig. 1). Thus, ductal fluid transport evoked by bombesin exhibits a greater dependency on extracellular calcium than that evoked by secretin.

The anion selectivity of ductal fluid secretion

Figure 2 shows that bromide, iodide and nitrate were all as effective as chloride in supporting fluid secretion measured in the presence of secretin, whereas gluconate and thiocyanate caused a marked inhibition of fluid transport. From these data the anion selectivity of fluid secretion from ducts stimulated with secretin can be calculated as: $Br^- = I^- = NO_3^- = Cl^-$ (1·0) $\gg$ thiocyanate = gluconate (0·3). Note that the ductal epithelium was still capable of secreting some fluid even when all the extracellular chloride had been replaced with gluconate, which is a large, impermeant anion.

In contrast to the results obtained with secretin, when bombesin was the stimulant only bromide could fully substitute for chloride in supporting fluid transport (Fig. 3). Iodide and nitrate acted as partial substitutes for chloride, whereas with thiocyanate and gluconate there was a marked inhibition of fluid

secretion (Fig. 3). Thus, the anion selectivity of fluid secretion from ducts stimulated with bombesin is: $Br^- = Cl^-$ (1·0) > $I^- = NO_3^-$ (0·6) > thiocyanate = gluconate (~ 0·3). Since fluid secretion evoked by bombesin is quite sensitive to extracellular calcium removal (Fig. 1), the inhibitory effect of gluconate replacement might in

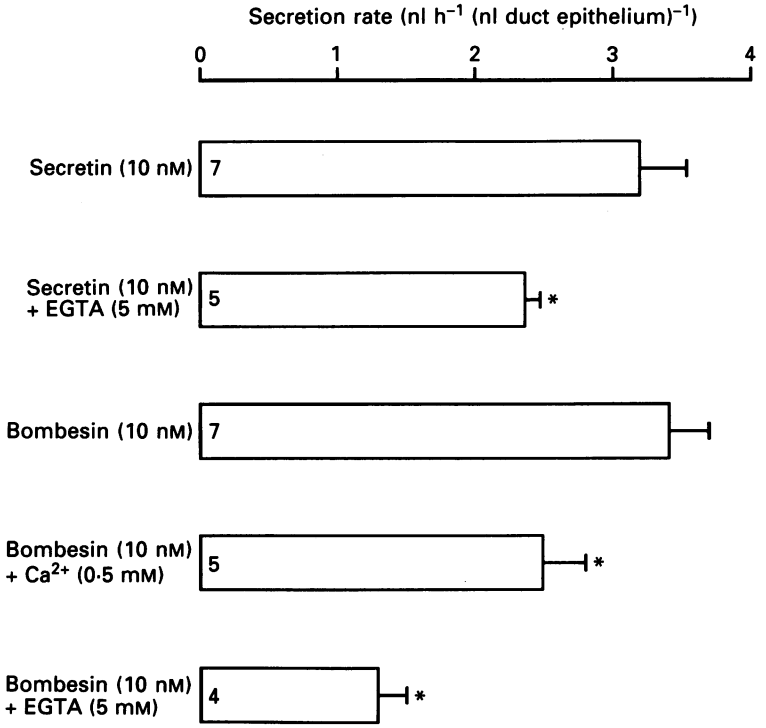

Fig. 1. The effect of a nominally Ca^{2+}-free buffer containing 5 mM-EGTA on fluid secretion from isolated interlobular ducts stimulated with either secretin (10 nM) or bombesin (10 nM). Statistical difference from secretin alone or bombesin alone, $*P < 0·05$ (ANOVA and SNK range test). The number of ducts in each group is indicated on the left-hand side of the columns.

part be explained by the fact that this anion chelates calcium (Kenyon & Gibbons, 1977). To check for this, we measured fluid transport by ducts stimulated with bombesin and perifused with a gluconate-containing buffer in which the total calcium concentration had been raised to 12·5 mM, giving a free ionic calcium concentration of about 2·5 mM (see Table 1 in Kenyon & Gibbons, 1977). Under these conditions, the secretory rate was $0·93 \pm 0·23$ nl h^{-1} (nl duct epithelium)$^{-1}$ ($n = 5$), which is not significantly different from the value measured when the total calcium concentration was 2·5 mM ($0·72 \pm 0·24$ nl h^{-1} (nl duct epithelium)$^{-1}$, $n = 5$; Fig. 3). This implies that the inhibitory effect of gluconate on fluid secretion measured in the presence of bombesin cannot be explained by extracellular calcium chelation.

Taken together, the results of these anion-replacement experiments point to a difference in the anion selectivity of fluid transport by pancreatic ducts stimulated

with bombesin and secretin. In particular, iodide and nitrate act as complete substitutes for chloride when secretin is the stimulant, but reduce fluid secretion evoked by bombesin.

The effects of SITS on ductal fluid secretion

Figure 4 compares the effect of the anion exchange inhibitor SITS (Knauf, 1979) on ductal fluid transport in the presence of secretin and bombesin. Whereas the

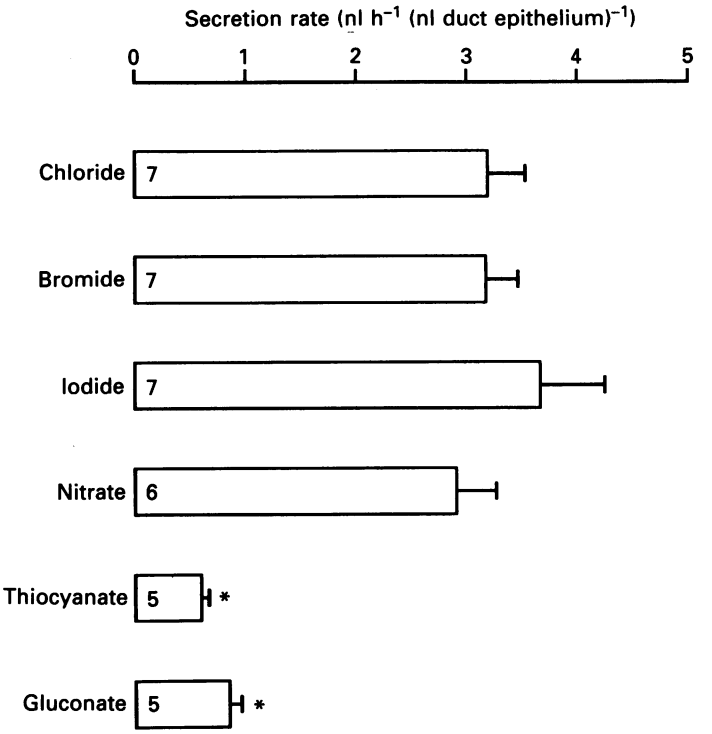

Fig. 2. The effect of various anions on fluid secretion from isolated interlobular ducts stimulated with secretin (10 nM). Statistical difference from the response obtained in the presence of chloride, $*P < 0{\cdot}05$ (ANOVA and SNK range test). The number of ducts in each group is indicated on the left-hand side of the columns.

response to secretin was unaffected by 1 mM-SITS, this concentration of the disulphonic stilbene reduced fluid transport measured in the presence of bombesin by 61%.

The effects of barium and TEA on ductal fluid secretion

In these experiments we employed the K^+ channel blockers, barium and TEA (Latorre & Miller, 1983), to test whether K^+ efflux across the basolateral membrane of the duct cell might play a role in fluid transport. Figure 5 shows that 3 mM-barium

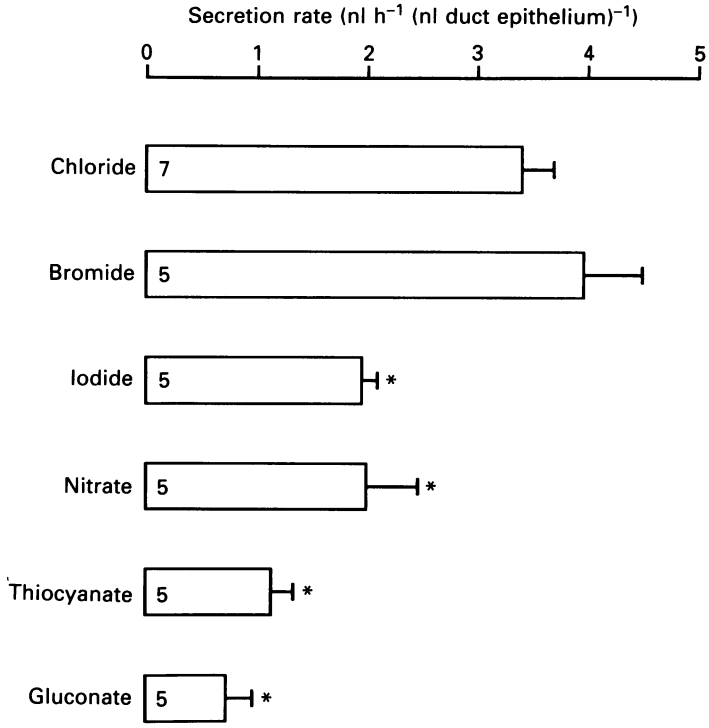

Fig. 3. The effect of various anions on fluid secretion from isolated interlobular ducts stimulated with bombesin (10 nM). Statistical difference from response obtained in the presence of chloride, $*P < 0{\cdot}05$ (ANOVA and SNK range test). The number of ducts in each group is indicated on the left-hand side of the columns.

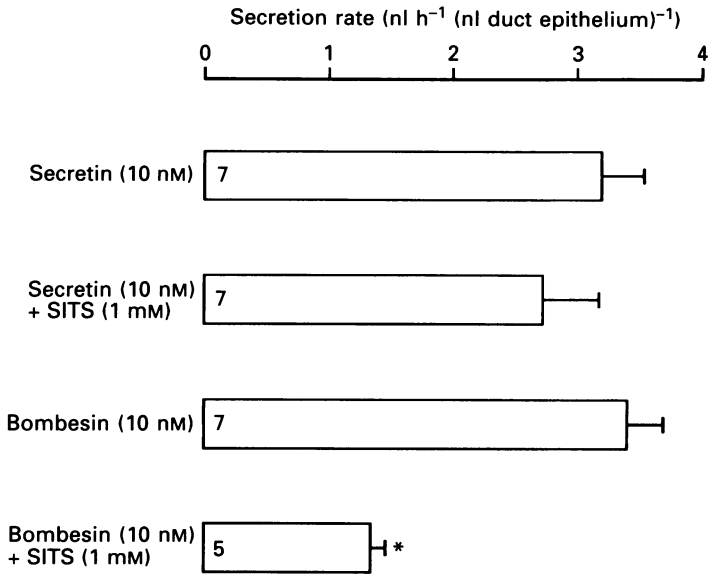

Fig. 4. The effect of SITS (1 mM) on fluid secretion from isolated interlobular ducts stimulated with either secretin (10 nM) or bombesin (10 nM). Statistical difference from bombesin alone, $*P < 0{\cdot}05$ (ANOVA and SNK range test). The number of ducts in each group is indicated on the left-hand side of the columns.

and 10 mM-TEA reduced fluid secretion measured in the presence of secretin and bombesin by between 52 and 66%.

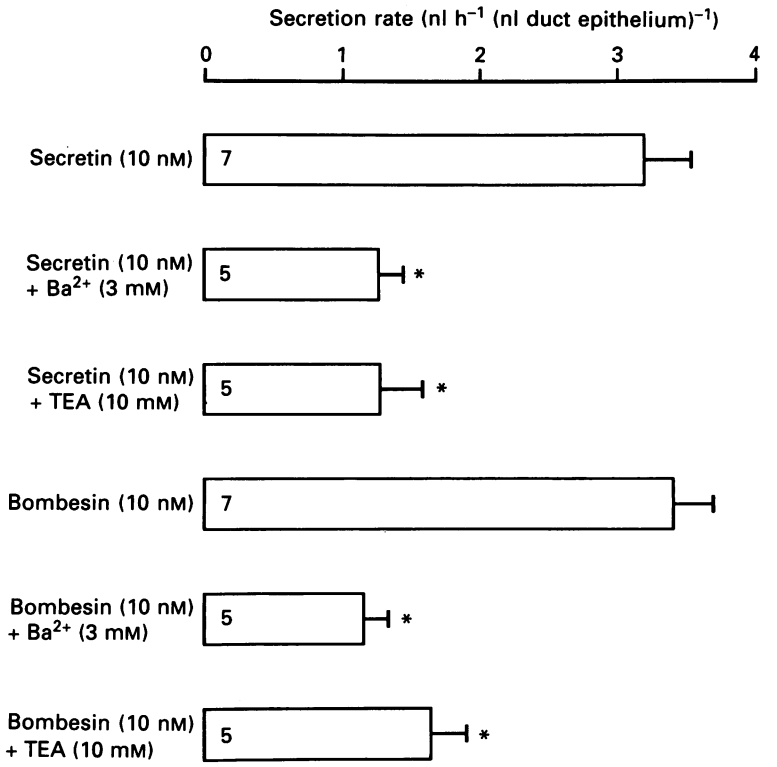

Fig. 5. The effect of barium (3 mM) and tetraethylammonium (TEA; 10 mM) on fluid secretion from isolated interlobular ducts stimulated with either secretin (10 nM) or bombesin (10 nM). Statistical difference from the response in the presence of secretin alone or bombesin alone, $*P < 0{\cdot}05$ (ANOVA and SNK range test). The number of ducts in each group is indicated on the left-hand side of the columns.

DISCUSSION

The ductal response to secretin

A cellular model for secretin-stimulated bicarbonate transport by pancreatic duct cells is shown in Fig. 6. This model is based on data derived from secretory studies on intact glands (reviewed in Case & Argent, 1989), together with recent electrophysiological and intracellular pH measurements made on small pancreatic ducts and cultured duct cells (Gray, Greenwell & Argent, 1988; Novak & Greger 1988*a*,*b*; Stuenkel, Machen & Williams, 1988; Gray, Harris, Coleman, Greenwell & Argent, 1989; Gray, Greenwell, Garton & Argent, 1990*a*; Gray, Pollard, Harris, Coleman, Greenwell & Argent, 1990*b*). Previously, it has been shown that fluid secretion evoked by secretin from the intact pancreas is quite resistant to extracellular calcium removal (Argent, Case & Scratcherd, 1973; Kanno & Yamamoto, 1977). This is usually taken to indicate that calcium is not an important intracellular messenger for the actions of this peptide on the duct cell, and our results

are consistent with this idea. On the other hand, there is good evidence to suggest that secretin receptors are coupled to adenylate cyclase, and that cyclic AMP is the main intracellular messenger for this hormone (Argent *et al.* 1986; Arkle *et al.* 1986; Case & Argent, 1989).

We found that chloride, bromide, iodide and nitrate were all equally effective in supporting fluid secretion from ducts stimulated with secretin, whereas thiocyanate and gluconate had a marked inhibitory effect. Assuming that the replacement anions are not toxic, the overall selectivity of the secretory mechanism should, in theory, identify the rate limiting step in anion transport. In the case of the duct cell, this could be either the Cl^-–HCO_3^- exchanger, or the chloride channel, both of which are located on the apical plasma membrane (Fig. 6). From Table 1, it can be seen that the anion selectivity of fluid secretion evoked by secretin most closely resembles the permeability sequence of the channel. Whereas bromide, iodide and nitrate are all as permeant as (or more permeant than) chloride in the channel, all these anions are transported more slowly than chloride on the exchanger.

Previously, anion selectivity sequences have been determined for fluid secretion evoked by secretin from the perfused cat and rat pancreas (Case, Hotz, Hutson, Scratcherd & Wynne, 1979; Evans, 1986). In these preparations, substitution of perfusate chloride with either glutamate, sulphate, methylsulphate or isethionate caused a marked inhibition of fluid transport stimulated by secretin, rather like the effect of gluconate and thiocyanate on isolated rat ducts. However, in contrast to our findings with isolated ducts, bromide, iodide, nitrate and thiocyanate all increased secretin-stimulated fluid secretion from the perfused rat pancreas (Evans, 1986); a discrepancy for which we have no ready explanation. In the cat, bromide and nitrate gave about the same secretory rate as chloride, whereas iodide inhibited fluid transport by 27% (Case *et al.* 1979).

The model shown in Fig. 6 predicts that ductal fluid secretion should be dependent on the presence of chloride ions, since this anion is required for cycling of the Cl^-–HCO_3^- exchanger located on the apical membrane of the duct cell. However, we found that some fluid was secreted by the ducts even after all extracellular chloride had been replaced by the impermeant anion gluconate. Similar results have been obtained using *in vitro* preparations of the cat, rat and rabbit pancreas (cat, Case *et al.* 1979; rat, Evans, 1986; rabbit, Rothman & Brooks, 1965; Kuijpers, Van Nooy, De Pont & Bonting, 1984*a*). This small, residual secretion which persists after chloride withdrawal may result from bicarbonate diffusing across the apical membrane of the duct cell via the channel, which we have recently shown to possess a small, but significant, permeability to this anion ($P_{Cl}/P_{HCO_3} \sim 0{\cdot}2$; Gray *et al.* 1990*b*). The same may also occur in mandibular acinar cells, which secrete a chloride-rich fluid when extracellular chloride is present, but a small volume of a bicarbonate-rich fluid when extracellular chloride is replaced by impermeant anions (Case, Hunter, Novak & Young, 1984).

When applied to the extracellular surface of the plasma membrane, disulphonic stilbenes inhibit anion exchangers (see Knauf, 1979), sodium-coupled anion transporters (see Preisig & Alpern, 1989), and also some types of epithelial chloride channels (see Bridges, Worrell, Frizzell & Benos, 1989). While inhibitory effects of SITS on fluid secretion evoked by secretin have previously been described in whole

gland preparations (Scratcherd & Hutson, 1982; Kuijpers, Van Nooy, De Pont & Bonting, 1984*b*; Evans, 1986), we found that 1 mM-SITS had no effect on fluid transport from isolated ducts stimulated with secretin. Again, we have no explanation for this discrepancy, except that SITS might inhibit fluid transport by

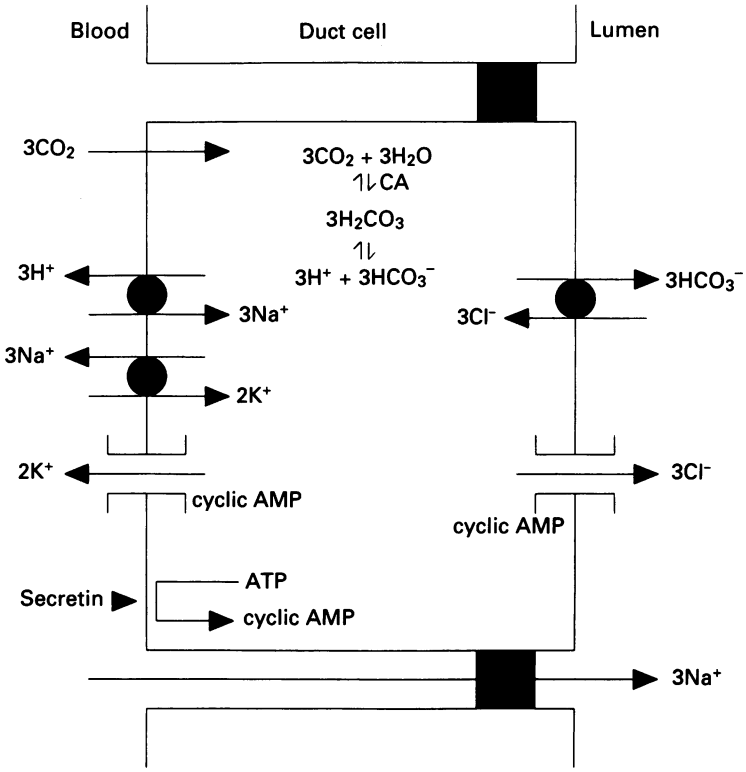

Fig. 6. Cellular model for pancreatic duct cell bicarbonate transport evoked by secretin. Carbon dioxide diffuses into the cytoplasm of the cell and is hydrated under the influence of carbonic anhydrase (CA) to carbonic acid. This dissociates to form a proton and a bicarbonate ion, and the proton is translocated back across the basolateral membrane on a Na^+–H^+ exchanger. Effectively, this is the active transport step for bicarbonate, the energy being derived from the sodium gradient established by the Na^+–K^+-ATPase. Bicarbonate ions are then thought to exit across the apical membrane of the duct cell on a Cl^-–HCO_3^- exchanger. The rate at which this exchanger cycles will depend on the availability of luminal chloride, which in turn depends on the open state probability of the apical chloride channel. This channel is activated following stimulation of the duct cell with secretin, presumably by a cyclic AMP-dependent phosphorylation event, and is an important control point in the secretory mechanism. Since bicarbonate exit at the apical membrane is electrogenic, outward current must flow across the basolateral membrane during secretion. Two-thirds of this current is accounted for by potassium efflux through a cyclic AMP-regulated maxi-K^+ channel, and one-third by cycling of the electrogenic sodium pump. The negative transepithelial potential is thought to drive sodium and potassium diffusion into the lumen via a cation selective paracellular pathway. Water follows the movement of ions by osmosis.

acini if these cells were also stimulated by secretin in the intact gland. Previously, SITS has been shown to inhibit the fluid secretion evoked by caerulein (Seow, Lingard & Young, 1986), which must originate from acinar cells in the rat pancreas since this peptide has no effect on isolated ducts (Argent *et al.* 1986). Because of its charged sulphonic acid residues, SITS is unlikely to enter the duct cells (Maddy,

TABLE 1. Comparison of the anion sequences for fluid secretion from isolated pancreatic ducts, with the permeability sequence for the duct cell chloride channel, and the selectivity sequence of the red blood cell anion exchanger

Parameter	Anion sequence	Reference
Ductal secretion		
Secretin	$Br^- = I^- = NO_3^- = Cl^- \gg SCN^- = \text{gluconate}$	(this paper)
Bombesin	$Br^- = Cl^- > I^- = NO_3^- \gg SCN^- = \text{gluconate}$	(this paper)
Duct cell channel	$NO_3^- > Br^- \approx I^- \approx Cl^- \gg HCO_3^- \gg \text{gluconate} = SO_4^{2-}$	(Gray *et al.* 1990*b*)
Anion exchanger	$HCO_3^- > Cl^- \gg Br^- > F^- \gg I^- > SO_4^{2-} > HPO_4^{2-}$	(Knauf, 1979)
	$Cl^- \approx Br^- > I^- > NO_3^- > SCN^-$	(Wieth, 1970)

1964). Moreover, the inhibitory effect of SITS on anion transport in other epithelia has been shown to exhibit a sidedness, i.e. the compound is only effective when applied to one side of the intact epithelium, suggesting that it does not cross tight junctions to any significant extent (Ehrenspeck & Brodsky, 1976; Greenwood, Bishop & Green, 1982; White & Imon, 1983). Therefore, our finding that SITS has no effect on ductal fluid transport stimulated by secretin, supports the idea that the transport mechanism activated by this peptide does not include a SITS-sensitive step at the basolateral membrane of the duct cell (Fig. 6).

As in chloride-secreting epithelia (Silva, Stoff, Field, Fine, Forrest & Epstein, 1977; Greger, Schlatter, Wang & Forrest, 1984), bicarbonate exit at the apical pole of the duct cell is thought to be electrogenic, with two-thirds of the current flow across the basolateral membrane being carried by K^+ efflux through regulated K^+ channels (Petersen, 1986; Fig. 6). Thus, secretin-stimulated fluid transport should be reduced if the basolateral K^+ channels are blocked, and this is exactly what we found when ducts were exposed to barium or TEA. Previously, it has been reported that these blockers (at the same concentrations we have employed) have no effect on fluid transport from the perfused rat pancreas stimulated with secretin, at least over a 10 min exposure period (Evans, Pirani, Cook & Young, 1986). It is possible that during our longer, 1 h exposure time, TEA and barium have toxic effects on the duct cells. However, it should be emphasized that maxi-K^+ channels which are stimulated by secretin, and blocked by TEA and barium, are present on the basolateral membrane of the duct cell (Gray *et al.* 1990*a*) and, furthermore, that basolateral application of barium, and to a lesser extent TEA, causes a depolarization of the duct cell membrane potential (Novak & Greger, 1988*a*).

The ductal response to bombesin

We found that fluid secretion evoked by bombesin was rather more sensitive to extracellular calcium depletion than that evoked by secretin. Although it is possible that this manoeuvre inhibits the binding of bombesin to its receptor, our finding is also consistent with calcium ions playing some role as an intracellular messenger in

the action of this peptide on the duct cell. However, using Fura-2 microspectrofluorimetry, we have not been able to detect any changes in intracellular calcium concentration following exposure of duct cells to this peptide (Evans, Ashton & Elliott, 1990). In the acinar cell, where its effects have been studied in some detail, bombesin is known to act via the inositol polyphosphate/diacylglycerol/Ca^{2+} pathway (Deschodt-Lanckman *et al.* 1976; Iwatsuki & Petersen, 1978; Pralong, Wollheim & Bruzzone, 1988). If the same is true in the duct cell, one would have to assume that the calcium signal is either very small, or perhaps restricted spatially to an area just below the plasma membrane, and thus not detected by the Fura technique.

When bombesin was the stimulant, only bromide could fully substitute for chloride in supporting ductal secretion, whereas iodide and nitrate caused a marked reduction in fluid transport. Inspection of Table 1 reveals that this anion sequence parallels the selectivity of the exchanger, and not the permeability of the channel, which was the finding with secretin. Another difference between the secretions evoked by these two peptides is their sensitivity to SITS; whereas this compound had no effect on the response to secretin, it markedly reduced bombesin-stimulated fluid transport. The simplest explanation for this finding is that a basolateral, SITS-sensitive, transport step is involved in the response to bombesin. Although it has been reported that basolateral SITS (1 mM) has no effect on the membrane potential of rat pancreatic duct cells (Novak & Greger, 1988*a*), these electrophysiological experiments were performed on unstimulated ducts in which the SITS-sensitive transport mechanism might be inactive.

Possible candidates for this SITS-sensitive transport step revealed by bombesin are the outwardly rectifying, and large conductance, voltage-dependent chloride channels that are present on the basolateral membrane of the duct cell (Argent, Gray & Greenwell, 1987; Gray *et al.* 1989). However, these channels are only rarely observed on intact cells and, furthermore, it is difficult to see how a basolateral chloride channel could be involved in electrogenic anion secretion. Alternatively, a SITS-sensitive, sodium-coupled transporter might be present on the basolateral membrane, although such a transporter has not been detected in microelectrode studies on unstimulated ducts (Novak & Greger, 1988*a*). Another possibility is that the basolateral membrane of bombesin-responsive cells contains an anion exchanger. From Fig. 7, it can be seen that moving the Cl^-–HCO_3^- exchanger from the apical to the basolateral membrane of the duct cell (where it would function in parallel with the Na^+–H^+ exchanger to accumulate chloride), would, in theory, convert the ductal epithelium into a chloride secreting tissue. Such a modification to the model would certainly explain the different effect of SITS on fluid secretion evoked by bombesin and secretin. Furthermore, bombesin-evoked chloride secretion (like secretin-evoked bicarbonate secretion) would be electrogenic, and should be blocked by barium and TEA, which is exactly what we found. A basolateral chloride accumulation step which involved parallel operation of Na^+–H^+ and Cl^-–HCO_3^- exchangers would also be consistent with our previous finding that bombesin-evoked fluid secretion is dependent on the presence of extracellular bicarbonate (Ashton *et al.* 1990).

In conclusion, we have provided evidence of differences in the calcium sensitivity, the anion selectivity, and in the SITS-sensitivity of fluid secretions evoked by

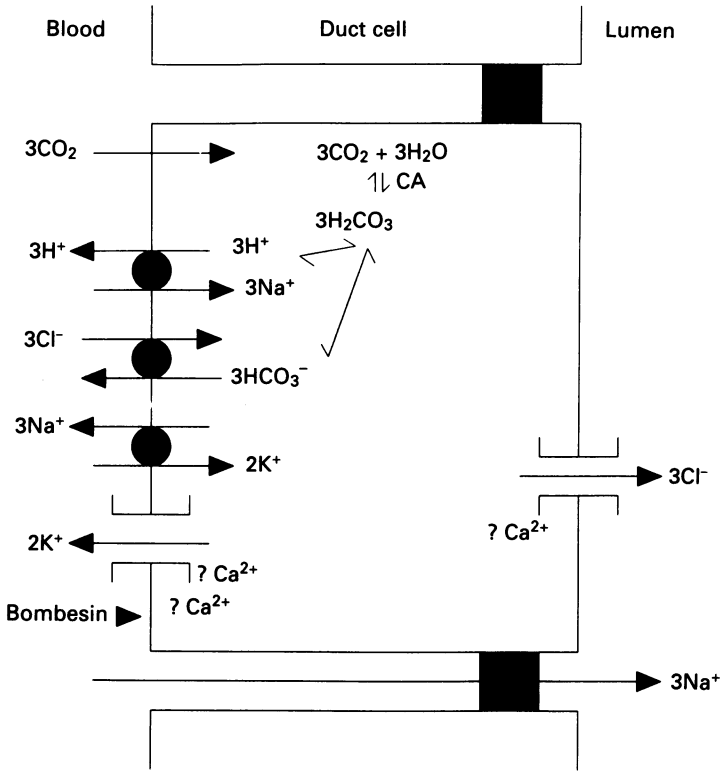

Fig. 7. Possible cellular model for pancreatic duct cell chloride transport evoked by bombesin. This model differs from the one shown in Fig. 6 in that the Cl^-–HCO_3^- exchanger is located on the basolateral membrane, and now functions in parallel with the Na^+–H^+ exchanger to accumulate chloride ions above electrochemical equilibrium. These chloride ions are then secreted across the apical membrane of the duct cell via the chloride channel. The other transport elements function as described in Fig. 6. The identity of the intracellular messenger(s) for ductal fluid transport stimulated by bombesin is uncertain; however, calcium ions may be involved.

secretin and bombesin from isolated rat pancreatic ducts. From this it seems likely that these two peptides utilize different intracellular signalling pathways and, furthermore, that they may activate different anion secretory mechanisms within the ductal epithelium.

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