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EFFECT OF VASOACTIVE INTESTINAL PEPTIDE, BOMBESIN AND SUBSTANCE P ON FLUID SECRETION BY ISOLATED RAT PANCREATIC DUCTS

BY N. ASHTON, B. E. ARGENT AND R. GREEN

From the Department of Physiological Sciences, University Medical Schools, Stopford Building, Manchester M13 9PT and Framlington Place, Newcastle upon Tyne NE2 4HH

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SUMMARY

1. We have used micropuncture techniques to study the regulation of fluid secretion by interlobular ducts isolated from the pancreas of copper-deficient rats.

2. Ducts isolated from different strains of Wistar rats exhibited quantitative differences in basal fluid secretion; however, secretion rates measured in the presence of secretin were similar.

3. Vasoactive intestinal peptide had no effect on fluid transport.

4. Bombesin stimulated fluid secretion, and this effect was abolished by removal of extracellular bicarbonate.

5. Substance P inhibited basal secretion, and that stimulated by bombesin and secretin. These inhibitory effects were partially reversed by spantide.

6. Substance P also inhibited fluid secretion stimulated by dibutyryl cyclic AMP and forskolin. This places the site of inhibition mediated by substance P at a point in the secretory mechanism distal to the generation of cyclic AMP.

7. We conclude that rat pancreatic duct cells possess receptors for bombesin and substance P, in addition to 'secretin-preferring' receptors. Since VIP had no effect on fluid transport, it is unlikely that 'VIP-preferring' receptors are present on rat duct cells.

INTRODUCTION

The exocrine pancreas consists of two epithelia; acinar cells, which secrete digestive enzymes together with a NaCl-rich fluid, and duct cells which produce a bicarbonate-rich secretion (for a review see Case & Argent, 1989). Until recently, it was generally assumed that physiological control of acinar secretion was mediated by cholecystokinin-like peptides and acetylcholine (released from the vagus), whereas secretin and vasoactive intestinal peptide (VIP) were the most important regulators of duct cell function. However, it is now clear that a number of other peptides, including bombesin and substance P (SP), can influence pancreatic enzyme and electrolyte secretion (Case & Argent, 1989).

Bombesin is a tetradecapeptide that was originally isolated from the skin of the European frogs *Bombina bombina* and *Bombina variegata variegata* (Anastasi,

Erspamer & Bucci, 1971), and which has subsequently been shown to stimulate pancreatic secretion in man, rats, dogs, guinea-pigs and pigs (man: Basso, Giri, Improta, Lezoche, Melchiorri, Percoco & Speranza, 1975; Delle-Fave, Annibale, de Magistris, Severi, Bruzzone, Puoti, Melchiorri, Torsoli & Erspamer, 1985; rat: Deschodt-Lanckman, Robberecht, De Neef, Lammens & Christophe, 1976; Linari, Linari & Lutoslawska, 1977; Iwatsuki & Petersen, 1978; Anderson & Dockray, 1988; dog: Konturek, Krol & Tasler, 1976; Singer, Niebel, Lamers, Becker, Vesper, Hartmann, Diemel & Goebell, 1981; Konturek, Tasler, Konturek, Cieszkowski, Szewczyk, Hladij & Anderson, 1989; guinea-pig: Padfield, Garner & Case, 1989; pig: Lilja, Greeley & Thompson, 1984; Knuhtsen, Holst, Jensen, Knigge & Nielsen, 1985). Bombesin is a potent stimulant of enzyme secretion in all these species; however, its effects on electrolyte secretion are variable. Whereas the dog pancreas secretes little fluid in response to bombesin stimulation (Konturek *et al.* 1989), the peptide is a powerful stimulant of water and electrolyte transport in the rat (Anderson & Dockray, 1988) and guinea-pig (Padfield *et al.* 1989).

The physiological mechanisms underlying these responses evoked by bombesin appears to differ between species. In the dog and pig, bombesin stimulates pancreatic secretion either through release of gastrin and CCK (Bertaccini, Erspamer, Melchiorri & Sopranzi 1974; Konturek *et al.* 1976; Lilja *et al.* 1984), or via a cholinergic mechanism (Taylor, Walsh, Carter, Wood & Grossman, 1979). On the other hand, in the rat, a direct effect of bombesin on the pancreas seems more likely (Anderson & Dockray, 1988). Bombesin receptors have been identified on a cell line derived from rat acini (Logsdon, Zhang, Guthrie, Vigna & Williams, 1987), and exposure of rat acinar cells to bombesin causes membrane depolarization and a reduction in input resistance (Iwatsuki & Petersen, 1978). However, nothing is known about the effect of bombesin on duct cells.

The undecapeptide SP was first detected in the brain and digestive tract by Euler & Gaddum (1931), and receptors for this peptide have been identified on guinea-pig pancreatic acinar cells (Sjödin, Brodin, Nilsson & Conlon, 1980). Furthermore, SP has been shown to increase basal pancreatic fluid and amylase secretion in the dog (Starke, Lembreck, Lorens & Weiss, 1968; Konturek, Jaworek, Tasler, Cieszkowski & Pawlik, 1981; Iwatsuki, Yamgishi & Chiba, 1986), rat and mouse (Katoh, Murai & Nonoyama, 1984), but to inhibit secretin-stimulated secretion in the dog (Konturek *et al.* 1981; Iwatsuki *et al.* 1986) and rat (Katoh *et al.* 1984).

The main aim of this study was to establish whether bombesin and SP can directly modulate fluid secretion by pancreatic duct cells. Because experiments on intact glands cannot distinguish between direct effects of these peptides on the ductal epithelium, and indirect effects mediated via the blood and/or nerve supply to the gland, we have studied their actions on fluid transport by isolated pancreatic ducts. Our results indicate that bombesin stimulates fluid secretion, whereas substance P has an inhibitory effect. We also show that VIP, which belongs to the same peptide family as secretin, has no effect on ductal fluid transport in the rat.

METHODS

Animals

Most experiments were performed on male Wistar rats (125–150 g) obtained from the Manchester University breeding colony; however, we occasionally purchased animals from Bantin & Kingman Ltd (Grimston, Aldbrough, UK). Both groups of rats 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 while the duct cells remain 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 is potentially harmful digestive enzymes is markedly reduced.

Isolation and culture of interlobular ducts

Copper-deficient rats were killed by cervical dislocation and the pancreas 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 typical of ducts within the intact pancreas of copper-replete rats (Argent *et al.* 1986; Arkle *et al.* 1986).

Solutions

The standard perifusion fluid for the micropuncture experiments was a Krebs–Ringer bicarbonate buffer which 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. To test for leaks in the ductal epithelium, [^{3}H]inulin was added to the perifusion buffer at a final concentration of 20 μCi ml^{-1}. In some experiments, bicarbonate, ions were omitted from the standard buffer and replaced with 10 mM-HEPES and 15 mM-NaCl. The pH of this bicarbonate-free solution was adjusted to 7·4 with sodium hydroxide, and it was gassed with 100% O_2.

Bombesin, SP, secretin, VIP, forskolin and dibutyryl cyclic AMP were all made-up as concentrated stocks in bicarbonate-free perifusion fluid containing 5% (w/v) bovine serum albumin. Stock solutions were infused into the tissue bath at a rate calculated to produce the required final concentration of the test substance. Albumin alone had no effect on ductal fluid transport.

Micropuncture experiments

These experiments were carried out as described by Argent *et al.* (1986), except that we used a Nikon Diaphot-TMD inverted microscope, a tissue bath which had a volume of 1 ml, and a perifusion flow rate of 2 ml min^{-1}. Secretion rates are expressed as nl h^{-1} (nl duct epithelium)$^{-1}$ and only one fluid collection was made from each duct.

We checked for damage-induced leakage around the micropuncture site by adding [^{3}H]inulin to the perifusion buffer and collecting fluid from ducts stimulated with 10^{-8} M-secretin (Argent *et al.* 1986). Contamination of the collected fluid by [^{3}H]inulin was not detected. Empirical calculations showed that it would have been possible to detect contamination when this exceeded 0·1 nl h^{-1} (nl duct epithelium)$^{-1}$.

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, SP, spantide, dibutyryl cyclic AMP, forskolin and bovine serum albumin (fraction V) were all obtained from Sigma. Pure natural porcine secretin and VIP were purchased from Professor V. Mutt, Karolinska Institutet, Stockholm, Sweden. [^{3}H]Inulin (1–5 Ci $mmol^{-1}$) was obtained from Amersham International plc. All other chemicals were of the highest purity available.

Statistical analyses

All values are expressed as the mean ± standard error of the mean (n = number of observations). Statistical comparisons were made using Student's unpaired t test, and differences were considered significant at the 5% level.

RESULTS

Basal secretion and the effect of secretin

Our initial study of fluid transport by isolated pancreatic ducts was carried out using Wistar rats purchased from Bantin & Kingman Ltd (Argent *et al.* 1986). Figure 1 shows that ducts isolated from these animals had a much lower basal secretion rate compared to ducts isolated from Wistar rats bred at Manchester University. However, there was no significant difference in the secretory response to 10^{-8} M-secretin (Fig. 1).

We checked that the elevated basal rate in the Manchester group was a true secretory effect, rather than a leak artifact caused by damage to the epithelium at the micropuncture site, by repeating the experiments at room temperature, and by removing bicarbonate ions from the perifusion buffer. Neither of these manoeuvres should affect a leak artifact; however, they reduced basal fluid transport by 60 and 38% respectively (Fig. 1). Furthermore, [^{3}H]inulin added to the extracellular solution bathing ducts isolated from Manchester rats was not detected in luminal fluid. Taken together, these results suggest that there may be quantitative variations in basal, but not secretin-stimulated, fluid secretion between ducts isolated from different strains of Wistar rats. All further experiments were performed on ducts isolated from Manchester University rats.

Effect of VIP

Figure 1 shows that VIP (10^{-8} M) had no effect on fluid transport.

Effect of bombesin

Bombesin increased fluid secretion from isolated ducts, and the dose–response curve for this effect is shown in Fig. 2. Fluid transport was significantly increased by 10^{-10} M-bombesin, and the dose required for a half-maximal response was about 8×10^{-10} M (Fig. 2). The maximal effect was obtained with 10^{-8} M-bombesin, which increased fluid transport 2·4-fold above the basal level; a response equivalent to that obtained with 10^{-8} M-secretin (compare Figs 1 and 2). Replacing bicarbonate ions in the perifusion buffer with HEPES greatly attenuated bombesin-stimulated fluid transport (Fig. 2). Indeed, the secretion rate measured under these conditions was comparable with that observed in a bicarbonate-free buffer alone (Fig. 2).

Effect of substance P

Substance P (SP) was a potent inhibitor of basal secretion, and of fluid secretion stimulated by secretin and bombesin. Figure 3 shows that the effect of SP on basal secretion was dose dependent; at 10^{-10} M-SP the inhibition was 54% and at 10^{-8} M-SP, 92%. Figure 3 also shows that these inhibitory effects of SP on basal secretion

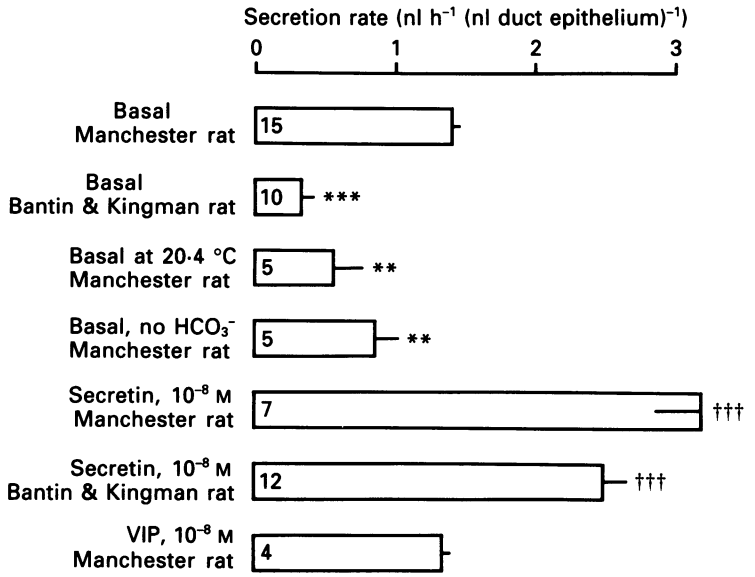

Fig. 1. Basal and VIP- and secretin-stimulated fluid secretion from isolated interlobular ducts. Rats were obtained from either the Manchester University breeding colony or from Bantin & Kingman Ltd. Statistical difference from basal secretion in Manchester rats: $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$; and from basal secretion in Bantin & Kingman rats: $\dagger\dagger\dagger P < 0{\cdot}001$. The number of ducts in each group is indicated at the left of the columns.

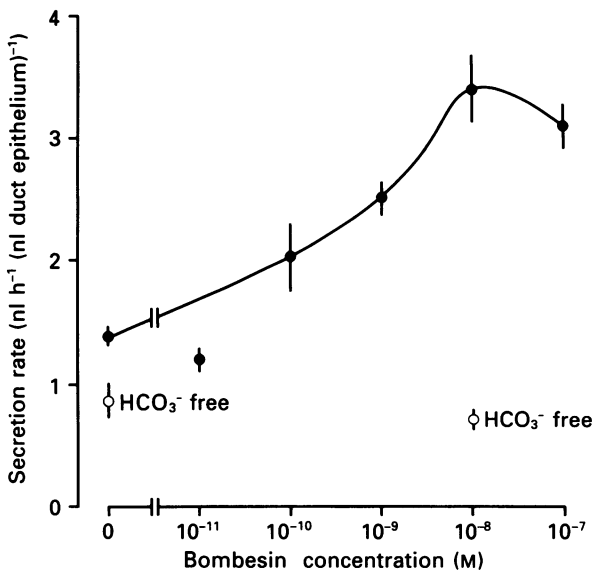

Fig. 2. Dose–response curve for bombesin-stimulated fluid secretion from isolated interlobular ducts (●), and the effect of extracellular bicarbonate on basal and bombesin-stimulated responses (○). Each point is the mean ± S.E.M. of four to fifteen observations.

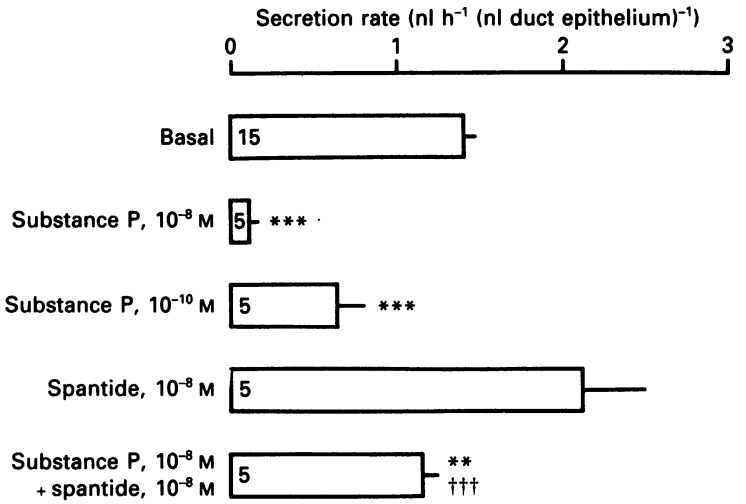

Fig. 3. Effect of substance P and spantide on basal fluid secretion from isolated interlobular ducts. Statistical difference from basal secretion: $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$; and from substance P (10^{-8} M): $\dagger\dagger\dagger P < 0{\cdot}001$. The number of ducts in each group is indicated at the left of the columns.

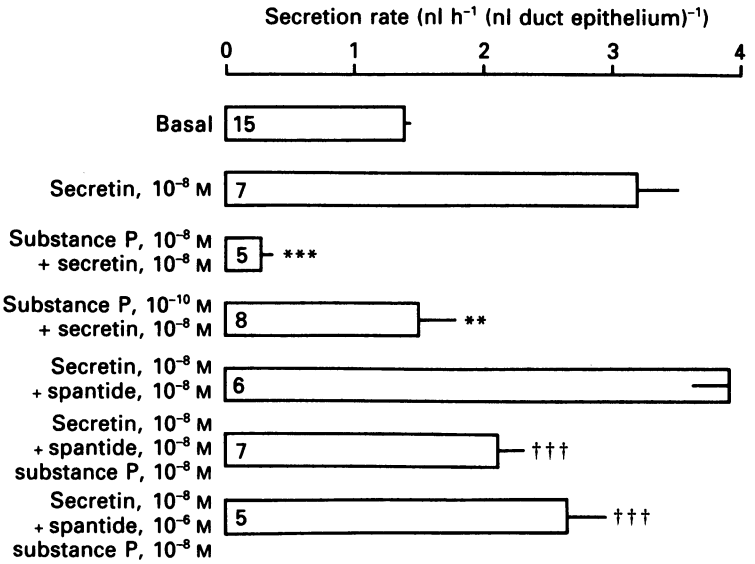

Fig. 4. Effect of substance P and spantide on secretin-stimulated fluid secretion from isolated interlobular ducts. Statistical difference from secretin (10^{-8} M) alone: $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$; and from secretin (10^{-8} M) plus substance P (10^{-8} M): $\dagger\dagger\dagger P < 0{\cdot}001$. The number of ducts in each group is indicated at the left of the columns.

were partially reversed by spantide, which is a SP receptor antagonist. Spantide (10^{-8} M) alone had no significant effect on basal fluid transport (Fig. 3).

Secretion rates measured in the presence of 10^{-10} M-SP and 10^{-8} M-secretin (Fig. 4) were not significantly different from basal, indicating that this concentration of SP

completely blocks fluid transport stimulated by secretin. Increasing the SP concentration to 10^{-8} M further reduced fluid secretion measured under these conditions to a value equivalent to about 20% of the basal response (Fig. 4). Again, these inhibitory effects of SP were partially reversed by spantide. In the presence of

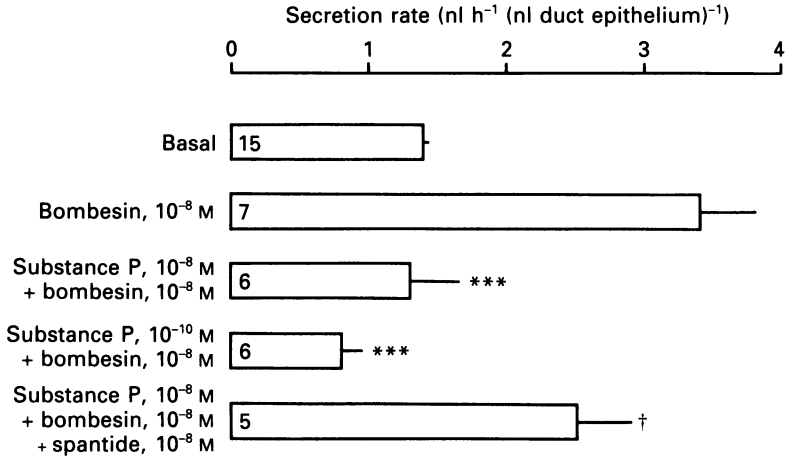

Fig. 5. Effect of substance P and spantide on bombesin-stimulated fluid secretion from isolated interlobular ducts. Statistical difference from bombesin (10^{-8} M) alone: ***$P < 0{\cdot}001$; and from bombesin (10^{-8} M) and substance P (10^{-8} M): †$P < 0{\cdot}05$. The number of ducts in each group is indicated at the left of the columns.

10^{-8} M-spantide, 10^{-8} M-SP reduced fluid transport stimulated by secretin by only 34%, which is about half the inhibitory effect observed with SP alone (Fig. 4). However, increasing the concentration of spantide to 10^{-6} M did not further reverse the inhibitory action of SP (Fig. 4). Figure 4 also shows that a combination of secretin (10^{-8} M) and spantide (10^{-8} M) produced a secretory response that was not significantly different from that obtained with secretin alone.

Figure 5 shows that in the presence of 10^{-10} M-SP, the secretory response to 10^{-8} M-bombesin was reduced by 72% to a value that is significantly lower than basal secretion ($P < 0{\cdot}01$). However, in contrast to the results obtained with secretin (Fig. 4), increasing the SP concentration to 10^{-8} did not further reduce fluid secretion measured in the presence of bombesin (Fig. 5). The inhibitory effect of SP (10^{-8} M) on fluid secretion stimulated by bombesin was also partially reversed by 10^{-8} M-spantide (Fig. 5).

Effect of substance P on fluid secretion stimulated by cyclic AMP

There is good evidence to suggest that cyclic AMP is an important intracellular messenger in ductal electrolyte secretion (Case & Argent, 1989), and we have previously shown that isolated ducts increase their cyclic AMP content when stimulated with secretin (Argent *et al.* 1986; Arkle *et al.* 1986), and that dibutyryl cyclic AMP stimulates fluid transport (Argent *et al.* 1986). In order to localize the site of SP-mediated inhibition within the secretory pathway, we tested the effect of this

peptide against fluid secretion stimulated by dibutyryl cyclic AMP, and also by forskolin which activates the catalytic subunit of adenylate cyclase.

Figure 6 shows that both dibutyryl cyclic AMP (2×10^{-4} M) and forskolin (3×10^{-6} M) were effective stimulants of fluid transport, increasing the basal rate of

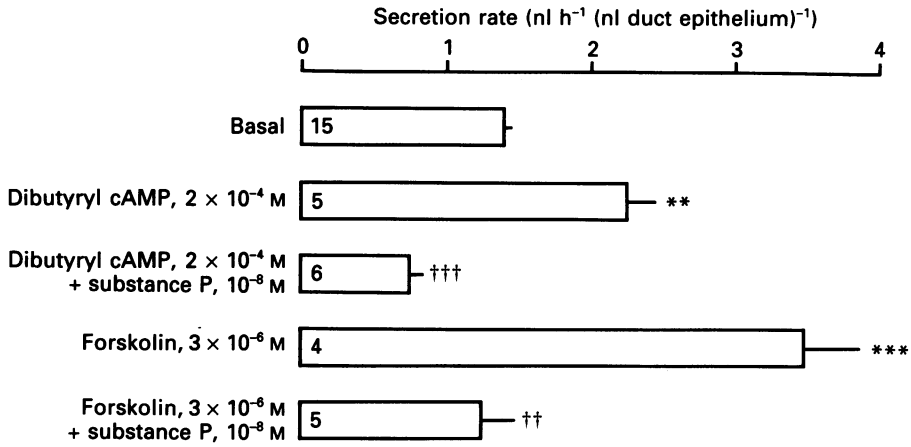

Fig. 6. Effect of substance P on fluid secretion from isolated interlobular ducts stimulated with either dibutyryl cyclic AMP or forskolin. Statistical difference from basal: $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$; and from either dibutyryl cyclic AMP alone or forskolin alone: $^{\dagger\dagger}P < 0{\cdot}01$, $^{\dagger\dagger\dagger}P < 0{\cdot}001$. The number of ducts in each group is indicated at the left of the columns.

secretion by 1·6- and 2·5-fold respectively. Figure 6 also shows that SP (10^{-8} M) significantly inhibited the secretagogue effect of both stimulants by about 65%.

DISCUSSION

Pancreatic ducts form a network of branching tubules which conduct digestive enzymes, secreted by acinar cells, into the duodenum. However, in addition to acting as a simple conduit, the ductal tree is an ion transporting epithelium which produces a bicarbonate-rich isotonic fluid (Case & Argent, 1989). This secretion flushes enzymes towards the gut, and is also partly responsible for neutralizing acid chyme which enters the duodenum from the stomach. Previously, we have shown that secretin is a potent stimulant of fluid secretion from isolated rat pancreatic ducts whereas caerulein, a cholecystokinin-like peptide which stimulates acinar fluid and enzyme secretion, has no effect (Argent *et al.* 1986).

Vasoactive intestinal peptide (VIP)

VIP is structurally related to secretin, and studies on guinea-pig acinar cells have shown that these peptides interact with two classes of receptors denoted 'secretin-preferring' and 'VIP-preferring' (Christophe, Conlon & Gardner, 1976). Our finding that 10^{-8} M-VIP had no effect on ductal fluid transport suggests that 'VIP-preferring' receptors are not present on rat pancreatic duct cells. This correlates with the weak effect of VIP on the secretion of fluid from the *in vivo* rat pancreas

(Dockray, 1973), and with the fact that doses of VIP in excess of 10^{-7} M are required to increase cyclic AMP levels in isolated duct fragments (Fölsch, Fischer, Söling & Creutzfeldt, 1980).

Bombesin

Bombesin-like immunoreactivity has been identified in the pancreas of several mammalian species including, in descending order of concentration, pig, man, rat, calf and guinea-pig (Ghatei, George, Major, Carlei, Polak & Bloom, 1983). Previously, bombesin has been shown to stimulate fluid secretion from the intact rat pancreas, but this action is usually ascribed to the well-known stimulatory effect of the peptide on pancreatic acini. Our results show that bombesin is as effective as secretin in stimulating ductal fluid secretion. Fluid transport from isolated ducts was significantly increased by 10^{-10} M-bombesin, and the dose required for a half-maximal response was about 8×10^{-10} M. This is similar to the effect of bombesin on rat pancreatic fragments where a dose of about 3×10^{-10} M gives a half-maximal effect on amylase secretion and ^{45}Ca efflux from acinar cells (Deschodt-Lanckman *et al.* 1976). The maximal effect on ductal secretion was obtained with 10^{-8} M-bombesin, which increased fluid transport 2·4-fold above the basal level.

Fluid secretion from the intact pancreas stimulated by secretin is dependent on the presence of bicarbonate ions in extracellular fluid (Case & Argent, 1989), and the same applies to isolated pancreatic ducts (Argent *et al.* 1986). This effect is usually taken to indicate that a supply of exogenous bicarbonate is required for the production of a bicarbonate-rich pancreatic juice (Argent *et al.* 1986; Case & Argent, 1989). Here we show that the stimulatory effect of bombesin on ductal fluid transport is also bicarbonate dependent. There are two possible interpretations of this result. Either the ducts are secreting a bicarbonate-rich fluid in response to stimulation with bombesin, or the peptide elicits secretion of a chloride-rich fluid, the production of which is bicarbonate dependent. Unfortunately, determining the composition of fluid secreted by isolated ducts is unlikely to be helpful in resolving this question, since the ductal epithelium contains a very active Cl^-–HCO_3^- exchanger which causes rapid equilibration of extracellular and intraluminal chloride concentrations (Argent *et al.* 1986). However, in pigs and guinea-pigs, bombesin stimulates bicarbonate secretion, as well as fluid and protein output, from the intact pancreas (Knuhtsen *et al.* 1985; Padfield *et al.* 1989).

Whatever the composition of ductal fluid secretions stimulated by secretin and bombesin, it is likely that these peptides utilize different intracellular signalling pathways. Secretin receptors are coupled to adenylate cyclase (Case & Argent, 1989), and ductal fluid transport can be stimulated by dibutyryl cyclic AMP and forskolin. Bombesin, on the other hand, stimulates enzyme secretion from acinar cells via the inositol polyphosphate/diacylglycerol/Ca^{2+} pathway (Deschodt-Lanckman *et al.* 1976; Iwatsuki & Petersen, 1978; Pralong, Wollheim & Bruzzone, 1988; Bruzzone, 1989). However, an increase in intracellular Ca^{2+} concentration is unlikely to be the mediator of bombesin effects on the duct cell since the peptide has no effect on this parameter in isolated pancreatic ducts (J. I. Gillespie, J. R. Greenwell & B. E. Argent, unpublished observations). Alternatively, bombesin could stimulate fluid transport either by enhancing cyclic AMP production via activation of protein

kinase C (Millar & Rozengurt, 1988), or by activation of the 5-lipoxygenase pathway (Bruzzone, 1989).

Substance P

Substance P-like immunoreactivity has been identified in the pancreas of the dog, cat, rat and mouse (Nilsson & Brodin, 1977), although it has not been localized around the ducts (Sharkey, Williams & Dockray, 1984). However, SP does inhibit secretin-stimulated fluid secretion from the dog and rat gland (Konturek *et al.* 1981; Katoh *et al.* 1984), and our results indicate that this effect can be ascribed to a direct action of SP on the ductal epithelium. We found that 10^{-10} and 10^{-8} M-SP reduced basal fluid secretion by 54 and 92% respectively, and that the same doses of SP completely blocked the responses to secretin and bombesin. These potent inhibitory effects of SP on ductal fluid transport contrast with its weak stimulant effect on amylase secretion from pancreatic acinar cells (Katoh *et al.* 1984; Jensen, Jones, Lu, Xu, Folkers & Gardner, 1984).

Because the inhibitory action of SP on fluid secretion was reversed by spantide, we conclude that it results from a direct interaction of SP with its receptors on the duct cell. A possible alternative explanation; that SP is an antagonist of secretin and bombesin receptors seems very unlikely since: (1) SP inhibited basal secretion from unstimulated ducts; (2) secretin does not compete for ^{125}I-physalaemin binding sites (SP receptors) in rat brain (Yachnis, Crawley, Jensen, McGrane & Moody, 1984); (3) neither secretin nor bombesin displaces ^{125}I-SP bound to guinea-pig acinar cells (Sjödin *et al.* 1980); (4) ^{125}I-Try^{4}-bombesin binding to rat brain is unaffected by SP (Yachnis *et al.* 1984). Furthermore, SP inhibited fluid secretion stimulated by dibutyryl cyclic AMP and forskolin. These observations place the inhibitory effect of SP at a point in the secretory mechanism down-stream from the generation of cyclic AMP; however, the link between SP receptor activation and inhibition of fluid secretion is unclear. Like bombesin, SP activates the inositol polyphosphate/diacylglycerol/Ca^{2+} pathway in acinar cells (Horstman, Takemura & Putney, 1988; Song, Iwashita, Noguchi & Konishi, 1988); however, differences must exist in the action of these two peptides on the duct cell in order to explain their divergent effects on fluid secretion.

We conclude that rat pancreatic duct cells possess receptors for bombesin and SP, in addition to 'secretin-preferring' receptors. Activation of bombesin receptors stimulates fluid transport, whereas activation of SP receptors causes a marked inhibition of basal and stimulated fluid secretion. Since VIP had no effect on fluid transport, it is unlikely that 'VIP-preferring' receptors are present in rat pancreatic ducts.

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