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PROSTAGLANDIN ACTION ON PANCREATIC BLOOD FLOW AND ON ELECTROLYTE AND ENZYME SECRETION BY EXOCRINE PANCREAS *IN VIVO* AND *IN VITRO*

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SUMMARY

1. Intra-arterial injection or infusion of prostaglandins E_1 and E_2 into anaesthetized cats caused a fall in arterial blood pressure, a reduction in pancreatic blood flow and an inhibition of secretin-stimulated pancreatic electrolyte secretion. In some experiments these effects were preceded by a transient increase in blood flow and secretion.

2. The fall in blood pressure and reduction in blood flow, but not the inhibition of secretion, were much less marked following administration of the α-adrenergic blocking agent phenoxybenzamine.

3. Prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ caused only a slight reduction in blood pressure and had very little effect on pancreatic blood flow or electrolyte secretion.

4. Addition of prostaglandins to the perfusate of the saline-perfused cat pancreas stimulated electrolyte secretion, with $E_1 = E_2 \gg F_{1\alpha} = F_{2\alpha}$. This stimulatory action was markedly potentiated by theophylline.

5. Enzyme secretion was not stimulated by any of the prostaglandins, even in the presence of theophylline.

6. It is concluded that prostaglandins can stimulate electrolyte transport by exocrine pancreas, perhaps through a mechanism involving adenylate cyclase, but that *in vivo* this action is masked by a secondary inhibition resulting either from vasoconstriction, or from the libration of an antisecretory agent, or both.

INTRODUCTION

A number of unifying schemes have been suggested to explain the physiological role and mode of action of the prostaglandins. One proposal, that they may form an intracellular regulating system controlling the

duration of hormone action, arose from the observed release by catecholamines and adrenocorticotrophic hormone of prostaglandins whose action is antagonistic to that of the hormones (Ramwell & Shaw, 1967). Since these hormones apparently act by modifying the intracellular concentration of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) (Robison, Butcher & Sutherland, 1968), Bergström (1967) speculated that, as a side reaction, the cyclic AMP so formed might activate lipase (as it is known to do in adipose tissue, Butcher, Ho, Meng & Sutherland, 1965, and liver, Claycomb & Kilsheimer, 1969) to produce fatty acids – the precursors of prostaglandins. The prostaglandins so formed could then regulate the accumulation of cyclic AMP in the cell by a feed-back mechanism influencing adenylate cyclase (the enzyme responsible for the synthesis of cyclic AMP from adenosine triphosphate).

A number of authors have observed that gastric acid secretion, stimulated by food, 2-deoxyglucose, histamine or gastrin, is inhibited by prostaglandin E_1 (PGE_1), both *in vivo* (Robert, Nezamis & Phillips, 1967, 1968; Lippman, 1968; Shaw & Ramwell, 1968; Jacobson, 1970 and Nezamis, Robert & Stowe, 1971) and *in vitro* (Way & Durbin, 1969) and it is tempting to postulate that such inhibition is the result of the feed-back mechanism envisaged above.

During an investigation into the actions of dibutyryl cyclic AMP and methyl xanthines on exocrine secretion from the perfused cat pancreas, a delayed inhibition of electrolyte secretion was observed with high doses of theophylline (Case & Scratcherd, 1972) (theophylline inhibits cyclic 3′,5′-nucleotide phosphodiesterase, the enzyme responsible for the breakdown of cyclic AMP, and thus allows accumulation of cyclic AMP within the cell). It seemed possible that this inhibition was the result of prostaglandin formation (Scratcherd & Case, 1972). Support for such a hypothesis has been presented by Rudick, Gonda, Dreiling & Janowitz (1971) who demonstrated that PGE_1 inhibits pancreatic secretion from dogs with chronic duodenal fistulae. However, the experiments described here suggest that this hypothesis is untenable. While an inhibitory action of PGE_1 and other prostaglandins on pancreatic electrolyte secretion was observed *in vivo*, stimulation was observed *in vitro*.

METHODS

For *in vivo* experiments, cats (1·5–3·0 kg) were denied food 18 hr before the experiment and were anaesthetized either with Nembutal (60 mg/kg, I.P.) or with chloralose (37·5 mg/kg) and urethane (450 mg/kg) following initial inductions with ether. After the splanchnic nerves had been sectioned extraperitoneally, a mid-line abdominal incision was made, through which the pylorus was ligated and the pancreatic duct cannulated at the point where it pierced the duodenal wall, the cannula being secure by a ligature which also occluded the bile duct. In some experiments the

vagi were sectioned in the neck. Prostaglandins, because of their rapid inactivation by liver and lungs (Ferreira & Vane, 1967), were injected into the aorta at a point above the exit of the coeliac axis through a fine polyetheylene tube inserted via the femoral artery. Carotid arterial blood pressure was monitored using a transducer (S.E. Laboratories, model 4–82) and ultra-violet oscillograph (S.E. Laboratories, model 3006). Either of two indices of pancreatic blood flow was measured: direct observation of the venous outflow from the gland (after appropriate surgical isolation of the gland) or measurement of the electrical conductance between platinum electrodes positioned over the tail of the gland at a frequency of 1592 Hz. At this frequency most current flows through the extracellular fluid, including blood (Clark, Greenwell, Harper, Sankey & Scratcherd, 1967), so that an increase occurs on vasodilatation and vice versa. Details of both these methods, with a justification of the latter, have been published (Barlow, Greenwell, Harper & Scratcherd, 1971).

For *in vitro* experiments, the pancreas was isolated from anaesthetized cats and perfused with a balanced salt solution as previously described (Case, Harper & Scratcherd, 1968). Prostaglandins were injected or infused into the arterial supply of the gland.

Secretin was prepared by the method of Crick, Harper & Raper (1949) or was obtained as a gift from Professor Viktor Mutt, G. I. Hormones Laboratory, Karolinska Institutet, Stockholm. Prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ (referred to as PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ respectively) were generous gifts from Professor S. Bergström, Department of Chemistry, Karolinska Institutet, Stockholm and Dr John Pike, Upjohn Company, Kalamazoo, Michigan.

Samples of pancreatic juice were collected in tared tubes, weighed and stored overnight at 4 °C. Enzyme secretion was monitored by measuring amylase activity using the method of Nørby (Lagerlöf, 1942). Bicarbonate concentrations were measured using a Natelson microgasometer (Scientific Industries, Inc., model 600), chloride was determined potentiometrically using 0·05 M $AgNO_3$ (Sanderson, 1952), and sodium and potassium were measured by flame photometry (Evans Electroselenium, Ltd, Mark II).

RESULTS

Effects of prostaglandins in vivo. There is usually no basal pancreatic secretion in anaesthetized cats. To test for an inhibitory effect of prostaglandins, it was therefore necessary to initiate and maintain a submaximal secretion of water and electrolytes by infusing secretin intravenously. In eight experiments intra-arterial injections of PGE_1 or PGE_2 (0·5–10 μg) consistently inhibited such a secretion for approximately 20 min to an approximately equal extent (Fig. 1). In some experiments, inhibition was preceded by a phase of increased secretion lasting less than 5 min (Fig. 2). This stimulatory action was also occasionally observed when PGE_1 was injected in the absence of a background secretin stimulation.

In six experiments the same doses of $PGF_{1\alpha}$ and $PGF_{2\alpha}$ did not inhibit secretion, though $PGF_{1\alpha}$ was occasionally observed to have a slight stimulatory action (similar to, but smaller than, that described above) both in the presence and absence of submaximal secretin stimulation (Fig. 1).

In addition to these effects on secretion, intra-arterial injections of PGE_1 and PGE_2 caused arterial blood pressure to fall and pancreatic blood

flow to be reduced (Figs. 2 and 3). Vasomotor effects in the pancreas were largely monitored using the conductance technique, as this involved virtually no surgical interference with the gland, and confirmed by direct observation (see Methods). The reduction in blood pressure was immediate. Recovery was slow (especially after vagal section) though sometimes (as in Fig. 2*A*) there was an initial phase of rapid recovery. The conductance record shows two phases: a transitory phase of increased conductance

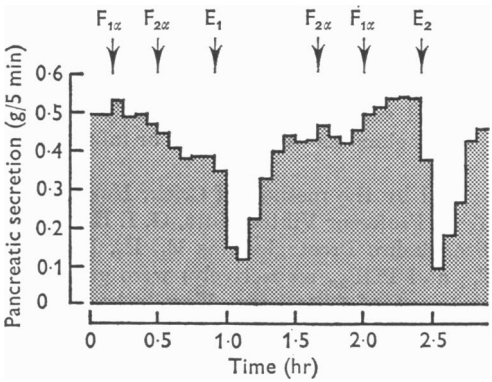

Fig. 1. Effect of prostaglandins on the rate of pancreatic secretion *in vivo*. The arrows indicate 5 μg pulses of the appropriate prostaglandin injected into the aorta above the coeliac axis. Secretion was stimulated maximally throughout by I.V. infusion of crude (Crick–Harper–Raper) secretin.

coinciding with the rapid fall in blood pressure, which was only infrequently observed and probably represents vasodilatation in the gland, followed by a prolonged phase of decreased conductance, indicating a decrease in blood content and/or flow. Following administration of the α-adrenergic blocking agent phenoxybenzamine (5 mg/kg) the fall in blood pressure was much less marked and recovery more rapid, while the phase of increased conductance was emphasized and that of decreased conductance virtually abolished. However, inhibition of pancreatic secretion still occurred (Fig. 2*B*).

Prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ caused smaller and transient falls in arterial blood pressure but did not reduce conductance; in fact a rise in pancreatic conductance was the most usual observation.

Effects of prostaglandins in vitro. The biphasic secretory response of the pancreas to intra-arterial injections of prostaglandins E_1 or E_2 suggested that these prostaglandins have two effects on pancreatic secretion: a direct stimulatory action on the cells of the exocrine pancreas and an inhibitory action which, at least in part, is indirect, resulting from a diminished blood

flow. They were therefore tested in the isolated pancreas. In fifteen experiments, pulses (1–10 μg) or infusions (0·12 μg/min; approximately 3×10^{-7} M) of PGE_1 or PGE_2 always caused a stimulation of water and electrolyte

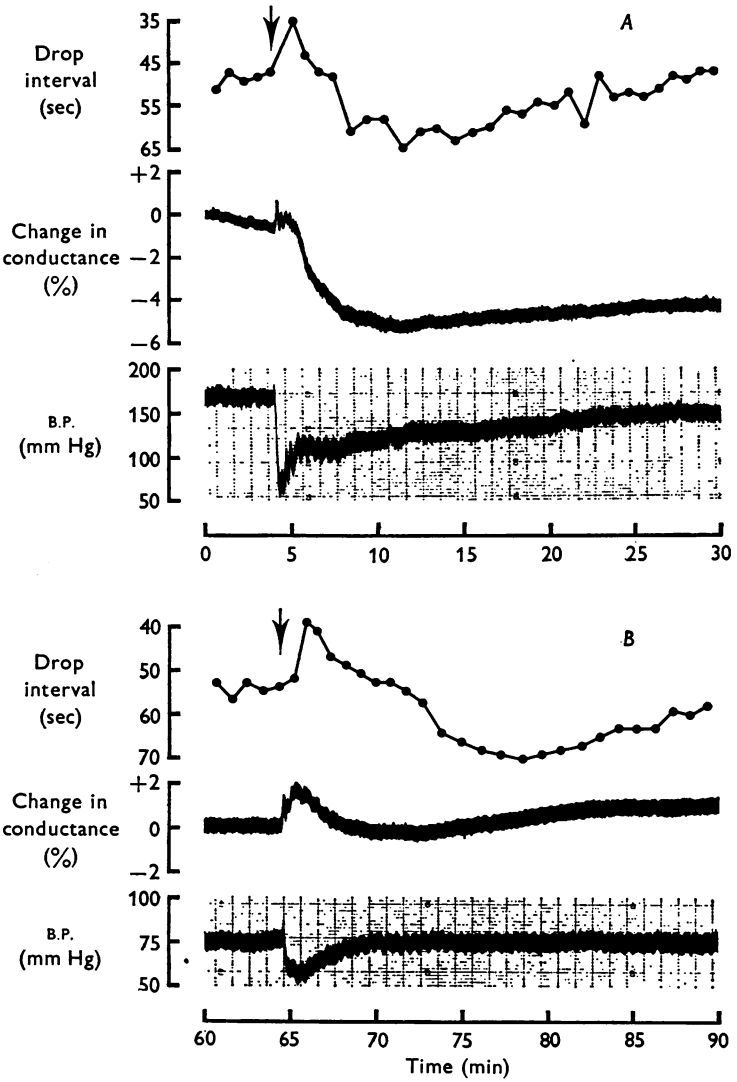

Fig. 2. Effect of PGE_1 on the rate of pancreatic secretion (expressed as the interval between drops of juice), electrical conductance across the tail of the pancreas and carotid arterial blood pressure in the anaesthetized cat. PGE_1 was given at the arrows as 5 μg pulses, injected into the aorta above the coeliac axis, (*A*) before and (*B*) after administration of the α-adrenergic blocking agent phenoxybenzamine. Secretion was stimulated submaximally throughout by the I.V. infusion of crude (Crick–Harper–Raper) secretin.

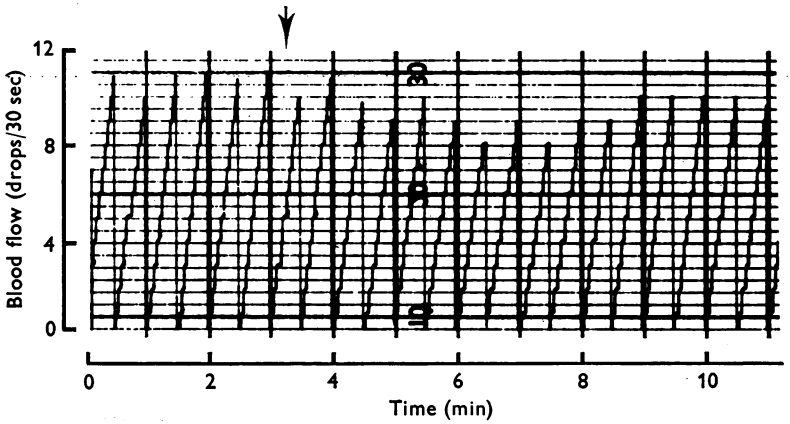

Fig. 3. Effect of PGE_1 on pancreatic blood flow. The pancreas was isolated as for *in vitro* experiments except that the blood circulation through the gland remained intact and was not replaced by saline perfusion. Blood from the gland was drained from the cannulated superior mesenteric vein (after ligation of the portal vein) and passed through a Lindgren drop counter before being returned to the femoral vein. This record shows the effect of a 5 μg pulse of PGE_1, injected into the aorta above the coeliac axis, on the venous outflow of the gland (drops/30 sec) measured in this way.

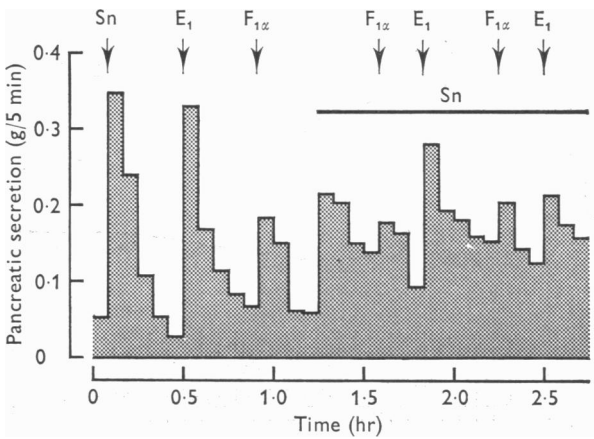

Fig. 4. Stimulation of pancreatic electrolyte secretion *in vitro* by prostaglandins. PGE_1 and $PGF_{1\alpha}$ (5 μg) were injected into the arterial supply of the perfused cat pancreas (at the times indicated by the arrows) before and during submaximal stimulation with crude (Crick–Harper–Raper) secretin (infused for the length of the horizontal bar). The first stimulation (Sn) is the result of injecting 50 μg of the same crude secretin. In this experiment, in which the effect of $PGF_{1\alpha}$ was the greatest observed, the responses to both PGE_1 and $PGF_{1\alpha}$ showed tachyphylaxis; this was not always the case.

secretion, whether tested alone or during submaximal secretin stimulation (Fig. 4). Prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ (ten experiments) frequently had a similar, though much smaller, effect. In some experiments the stimulatory effect of successive identical doses of prostaglandin became less, a phenomenon usually referred to as tachyphylaxis. Similarly, the secretory response to a constant infusion usually declined with time.

The electrolyte composition (Na^+, K^+, Cl^- and HCO_3^-) of juice secreted in response to prostaglandins E_1 and E_2 was similar to that of secretin stimulated juice secreted at the same rate.

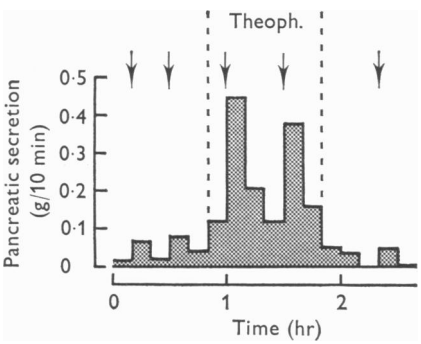

Fig. 5

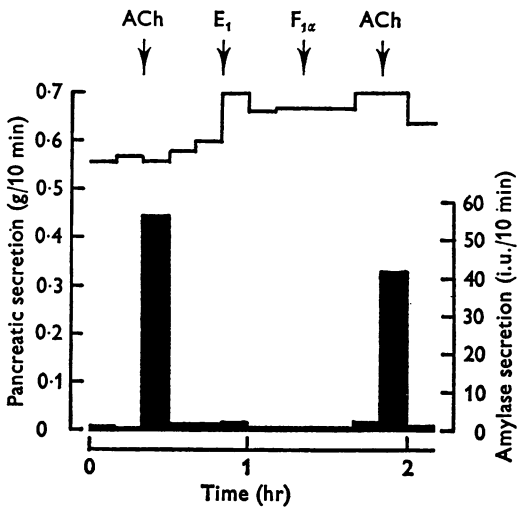

Fig. 6

Fig. 5. Potentiation of prostaglandin action by theophylline *in vitro*. PGE_1 (2·5 μg) was injected into the arterial supply of the perfused cat pancreas (at the times indicated by the arrows) before, during and after addition of theophylline (10^{-4} M) to the perfusion fluid. The gland was not stimulated by secretin. The stimulatory effect of theophylline alone has previously been described (Case & Scratcherd, 1972).

Fig. 6. Effect of prostaglandins on pancreatic enzyme secretion *in vitro*. PGE_1 and $PGF_{1\alpha}$ (5 μg) were injected into the arterial supply of the perfused cat pancreas (at the times indicated by the arrows), preceded and followed by a small injection of acetylcholine chloride (ACh, 200 ng), the stimulatory effect of which has been described elsewhere (Argent *et al.* 1971). Electrolyte secretion was stimulated maximally throughout by infusing pure (Mutt) secretin into the gland's arterial supply.

None of the prostaglandins had any consistent or significant effect on the rate of fluid perfusion through the gland.

The interaction of PGE_1 and theophylline. Evidence that secretin may stimulate ion transport in the pancreas through the intermediary of cyclic

AMP has previously been published (Case & Scratcherd, 1972; Case, Johnson, Scratcherd & Sherratt, 1972). In view of the interaction of prostaglandins with adenylate cyclase in many systems (see Introduction and Ramwell & Shaw, 1970), it seemed reasonable that the stimulatory effect of the prostaglandins described here involved cyclic AMP. This possibility was tested *in vitro* in five experiments by repeating injections of PGE_1, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ (2·5–10·0 μg) before, during and after addition of theophylline (10^{-3} or 10^{-4} M) to the perfusion fluid. Potentiation of the stimulatory response was consistently observed (Fig. 5), even when, with F series prostaglandins, the response to the prostaglandin alone was very small or even non-existent.

Effect of prostaglandins on enzyme secretion. When electrolyte and water secretion is stimulated from a previously resting cat pancreas (*in vivo* or *in vitro*), the secretion is rich in enzymes, whether the stimulus is secretin (Case, Harper & Scratcherd, 1969), dibutyryl cyclic AMP or theophylline (Case & Scratcherd, 1972), or prostaglandins (Scratcherd & Case, 1972). This so called 'wash-out' phenomenon results from a small but continuous basal secretion of enzymes into the duct system and is therefore a passive secretion; it cannot be used as evidence that any of these stimuli, including prostaglandins, actively stimulate enzyme secretion by the gland. To distinguish between passive and active enzyme secretion, in eight experiments prostaglandins were injected or infused into the arterial supply of the isolated pancreas secreting at high flow rates in response to secretin. In no instance was an increase in amylase activity observed with any of the four prostaglandins tested (Fig. 6).

DISCUSSION

The observations described above clearly demonstrate that prostaglandins E_1 and E_2 can both stimulate and inhibit pancreatic electrolyte and water secretion. Because these actions respectively are more pronounced *in vitro* and *in vitro* it is likely that stimulation is due to a direct effect of the prostaglandins on the gland, while inhibition probably results from an indirect effect. Prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ seem only to possess a slight stimulatory effect.

Indirect inhibition could originate in two ways: by the liberation of an agent with anti-secretory properties, or through vasoconstriction by limiting the supply of nutrients and stimulants (secretin) to the gland. Vasoconstriction in turn might be caused by the prostaglandins themselves, by nervous reflexes or by the release of vasoconstrictor agents. Certainly vasoconstriction does occur *in vivo* following injection of prostaglandins E_1 and E_2, as evidenced both by a decreased electrical conductance across the gland and by a reduced blood flow. However, a direct

constrictor action of prostaglandins on the vasculature of the pancreas is unlikely, as they had no effect on the perfusion rate in the isolated gland, suggesting that vasoconstriction represents part of the reflex homoeostatic response to the fall in arterial blood pressure observed in these experiments and previously well documented (see Bergström, Carlson & Weeks, 1968). PGE_1 can release catecholamines from the adrenal medulla in the dog, possibly through a pre-synaptic action (Kayaalp & Türker, 1967), though apparently not in the cat (Horton, 1963; Miele, 1969). PGE_1 can also stimulate the sympathetic nervous system in some tissues, including the cardiovascular system (Carlson & Orö, 1966, Bergström, Carlson & Orö, 1966; though it can also inhibit release of, and effector response to, noradrenaline in others; Hedqvist, 1970) and Bergström *et al.* (1968) have therefore suggested that biogenic amine liberation should always be considered when prostaglandins are injected into intact animals. The modifying effect of phenoxybenzamine on the observed cardiovascular responses to prostaglandins E_1 and E_2 certainly suggests that a part of the action of these prostaglandins is mediated through catecholamine release.

However, the persistence of secretory inhibition after phenoxybenzamine in spite of the reversal of the constrictor effect on the blood vessels is strikingly similar to the action of adrenaline and noradrenaline on the pancreas recently described by Barlow *et al.* (1971). The failure of phenoxybenzamine to prevent the inhibition of secretion by PGE_1 and PGE_2 does not therefore eliminate a direct action on the secretory cell of adrenergic inhibitors liberated by these compounds.

The transitory increase in secretion occasionally observed *in vivo* following prostaglandins E_1 and E_2 may have been caused either by an augmented supply of secretin to the gland, consequent upon the temporary increase in blood flow also observed to occur in these experiments, or by the direct stimulatory action of these prostaglandins on the pancreas (see below). Similar explanations may account for the very slight stimulation of secretion following prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$.

In the experiments of Rudick *et al.* (1971) using dogs with chronic duodenal fistulae, besides inhibiting electrolyte secretion, PGE_1 clearly had many extra-pancreatic effects, as indicated by the massive autonomic discharge (vomiting, defaecation and urination) observed with high doses, thus supporting the possibility of catecholamine release discussed above. The stimulation of enzyme secretion by PGE_1 described by these authors may also have been an indirect effect, dependent upon the increased autonomic discharge, since none of the prostaglandins tested in the isolated cat pancreas were capable of evoking enzyme secretion.

Whatever the cause of inhibition *in vivo*, there is no doubt that prosta-

glandins stimulate water and electrolyte secretion from the isolated gland. An action of prostaglandins on electrolyte transport was first described as occurring in toad bladder, where PGE_1 inhibits vasopressin-induced fluid movement (Orloff, Handler & Bergström, 1965; Eggena, Schwartz & Walter, 1970). An identical situation occurs in turtle bladder (Lipson & Sharp, 1971). PGE_1 also inhibits the action of vasopressin on isolated collecting tubules of rabbit kidney cortex, but in this tissue, unlike its action in the bladder, PGE_1 alone slightly increases net water transport (Grantham & Orloff, 1968). Similarly in isolated frog gastric mucosa PGE_1 inhibits gastrin-stimulated H^+ secretion (Way & Durbin, 1969; Shaw & Ramwell, 1969) while alone it is capable of eliciting secretion (Shaw & Ramwell, 1969). A similar dual effect of prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ occurs in the macromolecular secretion of the thyroid gland. Alone, these prostaglandins augment the release of ^{131}I, but they also reduce the secretion due to submaximal stimulation by thyroid stimulating hormone or long acting thyroid stimulator (Burke, 1970).

A situation more analogous to that in the pancreas is seen in the gut. Superior mesenteric arterial infusions of prostaglandins E_1, A_1 or $F_{2\alpha}$ induce a net secretion of water and electrolytes from jejunal loops of lightly anaesthetized dogs (Pierce, Carpenter, Elliott & Greenough, 1971), and prostaglandins E_1 and E_2 (at a concentration of $1{\cdot}5 \times 10^{-7}$ M) and some other prostaglandins (at higher concentration) increase short-circuit current across isolated guinea-pig ileal mucosa, probably by stimulating active Cl^- secretion (Kimberg, Field, Johnson, Henderson & Gershon, 1971).

In each of the tissues mentioned above the respective transport mechanism is stimulated by exogenous cyclic AMP or dibutryl cyclic AMP (bladder: Orloff & Handler, 1962; kidney: Grantham & Burg, 1966; gastric mucosa: Harris, Nigon & Alonso, 1969; thyroid: Willems, Rocmans & Dumont, 1970; gut: Field, 1971; pancreas: Case & Scratcherd, 1972). Where tested, the inhibitory effects of PGE_1 are not observed during cyclic AMP-stimulated transport (bladder: Orloff *et al.* 1965; kidney: Grantham & Orloff, 1968; gastric mucosa: Way & Durbin, 1969) indicating that PGE_1 acts by modifying adenylate cyclase activity. Indeed, adenylate cyclase preparations from a number of tissues, including gastric mucosa (Perrier & Laster, 1972) and gut (Kimberg *et al.* 1971) are stimulated by PGE_1. Although an effect of prostaglandins on pancreatic adenylate cyclase has not yet been described, the potentiation by theophylline of prostaglandin-stimulated electrolyte suggests that in this tissue also they may act through adenylate cyclase. The diverse effects (inhibition and stimulation) of prostaglandins in various tissues may be explained if, as some have suggested (e.g. Eggena *et al.* 1970), they act as partial agonists on a receptor functionally coupled with adenylate cyclase. Their effect in a given

tissue would then depend on the concentration of the prostaglandin and on the basal activity of adenylate cyclase.

The role of prostaglandins in pancreatic exocrine secretion is difficult to assess and will remain so until the effects of nervous and hormonal stimuli on prostaglandin formation and identity are known. Use of the saline-perfused pancreas could assist in solving this problem.

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