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CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE CONCENTRATION IN THE PANCREAS FOLLOWING STIMULATION BY SECRETIN, CHOLECYSTOKININ-PANCREOZYMIN AND ACETYLCHOLINE*

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SUMMARY

1. Following an I.V. injection of secretin into anaesthetized cats, the pancreatic cyclic AMP concentration rose within 30 sec and reached near-maximal values within 1 min. Pancreatic secretion began only after 45 sec. As secretion declined, the cyclic AMP concentration also fell. However, after 40 min, when secretion had ceased, the concentration again rose, reaching a maximum after about 80 min and returned to basal values within 140 min.

2. During secretin infusion the pattern of cyclic AMP changes was the same, except that the initial rise was maintained as long as secretin was infused.

3. Following either pancreozymin or acetylcholine, alone or superimposed on secretin stimulation, similar changes in cyclic AMP concentration were observed. However, the initial rise lasted only 30 sec, basal concentrations being approached within 1 min, and was accompanied by enzyme secretion. The concentration of cyclic AMP subsequently rose and fell again, in the absence of enzyme secretion, exactly as after secretin stimulation.

4. Similar observations were made using an isolated, saline-perfused preparation of the cat's pancreas.

* Throughout this paper cholecystokinin-pancreozymin will subsequently be referred to simply as pancreozymin.

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5. By using very low doses of pancreozymin it was possible to observe the first rise in cyclic AMP concentration in the absence of enzyme secretion. Similarly atropine, while blocking enzyme secretion, did not affect the rise in cyclic AMP concentration after acetylcholine. The second increase in concentration was never associated with secretion (it may have been connected with the synthesis of exportable enzymes by the gland).

6. While these observations suggest that cyclic AMP may be involved in the response of the pancreas to secretin, pancreozymin and acetylcholine, no simple relation exists between cyclic AMP concentration and secretion.

INTRODUCTION

In the previous paper (Case & Scratcherd, 1972) was outlined the development of the concept that cyclic adenosine 3′,5′-monophosphate (cyclic AMP) is an intracellular mediator in the action of many hormones. Evidence for the involvement of cyclic AMP in the action of secretin and pancreozymin was sought by testing two of four criteria proposed by Sutherland, Robison & Butcher (1968), namely: could the actions of these hormones be mimicked by exogenous cyclic AMP and be potentiated by methyl xanthines. The observations strongly supported cyclic AMP as a mediator in the action of secretin, but gave little support for a similar role in pancreozymin action (Case & Scratcherd, 1972).

It was therefore desirable to test the other criteria. The use of a sensitive enzymic assay for cyclic AMP (Johnson & Sherratt, 1970) allows cyclic AMP concentrations to be determined in very small samples of tissue. It was now possible to follow cyclic AMP concentrations in pancreatic tissue for many hours after stimulation by secretin, by pancreozymin or by acetylcholine, whilst simultaneously monitoring the physiological response to the hormone.

A preliminary account of this work has been published (Johnson, Sherratt, Case & Scratcherd, 1970).

METHODS

Experiments were performed on young, lean cats (weight: 1·5–2·3 kg, mean 1·75 kg), denied food for 18 hr before the experiment. The animals were anaesthetized with Nembutal (60 mg/kg, I.P.). 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.

Either one, or both, of the external jugular veins were cannulated with polyethylene tubing to allow secretin and pancreozymin to be administered alone, or together. Acetylcholine, because of its rapid destruction, was injected into the aorta at a point above the exit of the coeliac axis, by means of a long, thin, polythene cannula, inserted via the femoral artery.

To compare the behaviour of the gland *in vivo* and *in vitro*, two experiments were performed on a saline-perfused preparation of the pancreas, which has been described in the foregoing paper (Case & Scratcherd, 1972).

Samples of pancreatic tissue were taken from the whole of the gland (though generally from the body and tail region) in a random fashion. This precaution seemed to be of little importance, since the resting concentration of cyclic AMP varied little throughout the gland (see Fig. 1). After removal, tissue samples were immediately transferred to isopentane and the cyclic AMP concentration determined on a blind basis in a modified enzyme assay based on the conversion of phosphorylase *b* to phosphorylase *a* in the presence of purified phosphorylase *b* kinase and phosphorylase *b* kinase kinase. Phosphorylase *a* activity, which depends indirectly on the concentration of cyclic AMP present, was measured fluorimetrically with a coupled enzyme assay (Johnson & Sherratt, 1970). The method will be published in detail elsewhere (Johnson, M. & Sherratt, H. S. A., unpublished). Cyclic AMP concentrations are expressed as moles/kg wet wt. tissue except in the perfusion experiments where, because of oedema, concentrations are expressed as moles/kg protein (assayed by the method of Folin & Ciocalteu on part of each sample; Miller, 1959). Samples of pancreatic juice were collected in tared Pyrex tubes, weighed and stored overnight at 4° C. Amylase (EC 3.2.1.1.) activity was determined by the method of Nørby (Lagerlöf, 1942). Secretin and pancreozymin were prepared by the method of Crick, Harper & Raper (1949), though the results obtained using these crude preparations were checked using synthetic secretin (a gift from Dr Ondetti, Squibb Institute for Medical Research, New Brunswick, N.J., U.S.A.) and pure natural cholecystokinin-pancreozymin (generously donated by Professor Viktor Mutt, Department of Chemistry, Karolinska Institutet, Stockholm, Sweden).

RESULTS

Cyclic AMP concentration in resting pancreas. The cyclic AMP concentration in pancreatic tissue from sixteen anaesthetized cats was $2{\cdot}74$ $(\pm 0{\cdot}14) \times 10^{-7}$ mole.kg^{-1} (mean $\pm$ S.E. of mean). In a control experiment the resting concentration was followed for 3 hr (Fig. 1), when there was very little variation. This experiment also demonstrates the accuracy of the assay technique.

The effect of theophylline on this resting concentration was observed in three animals. Theophylline (10 mM in 0·14 M-NaCl) was infused at a rate of 1·5 ml. min^{-1}, after a priming dose of 10 ml. It caused a small flow of pancreatic juice. After 20 min infusion, the cyclic AMP concentration increased respectively from 3·20, 2·87 and $2{\cdot}35 \times 10^{-7}$ M.kg^{-1} to 5·80, 4·75 and $3{\cdot}70 \times 10^{-7}$ M.kg^{-1}.

Effect of secretin on cyclic AMP concentration. In seven experiments the effect of a single intravenous injection of 0·5 mg secretin on the cyclic AMP concentration in cat pancreas was remarkably similar. The most representative experiment is illustrated in Fig. 2. 30 sec after the secretin injection, the cyclic AMP concentration had risen by 140 %; after a minute it had risen by a total of 250 %, and was near maximum. After 5 min the concentration began to decline, reaching a lower limit (which in this

experiment was still above basal concentration) after approximately 40 min. In these experiments, secretion was never observed to occur until 45 sec after secretin injection, and in this laboratory has never been observed before 35 sec, so that the rise in cyclic AMP concentration undoubtedly occurred before secretion commenced. Following a single injection of secretin the secretion rate tends to decline in an exponential

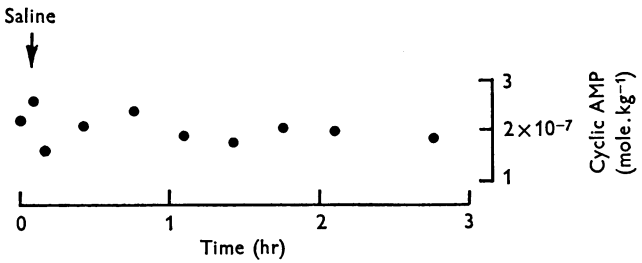

Fig. 1. Anaesthetized cat: control experiment. Effect of a single injection of saline (1·0 ml. 0·14 M NaCl, I.V.) given at the arrow, on the concentration of cyclic adenosine 3′,5′-monophosphate (●), expressed as mole.kg^{-1} wet wt. at the time indicated, in samples of pancreatic tissue taken randomly from the whole gland.

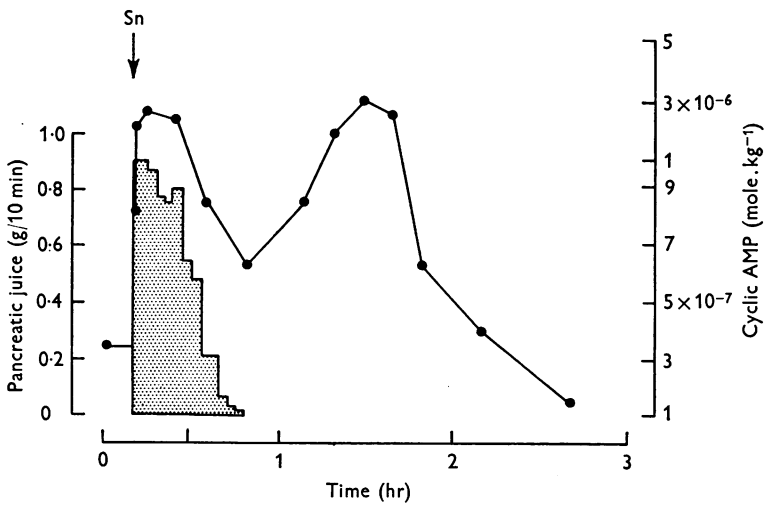

Fig. 2. Anaesthetized cat. Effect of a single injection of crude (Crick–Harper–Raper) secretin (0·5 mg, I.V.) given at the arrow, on pancreatic electrolyte secretion (stippled) and on the concentration of cyclic adenosine 3′,5′-monophosphate (●) in the pancreas, expressed as mole.kg^{-1} wet wt. tissue at the time indicated. In this and subsequent figures, the concentrations of cyclic adenosine 3′,5′-monophosphate at various times are joined by a line for ease of interpretation: it should not be inferred that the concentration of cyclic AMP at a given time necessarily falls on this line.

fashion (Clark, Greenwell, Harper, Sankey & Scratcherd, 1967), though this is not well illustrated in Fig. 2. Thus although the secretory rate usually followed the cyclic AMP concentration, this was not always so, since the maximal secretory rate was achieved almost immediately, whereas cyclic AMP did not reach maximal concentration for 5 min. However, over the next hour, these two parameters were completely dissociated; cyclic AMP concentrations rose again in the absence of pancreatic secretion, reaching a peak at 80–90 min. The maximal cyclic AMP concentration achieved

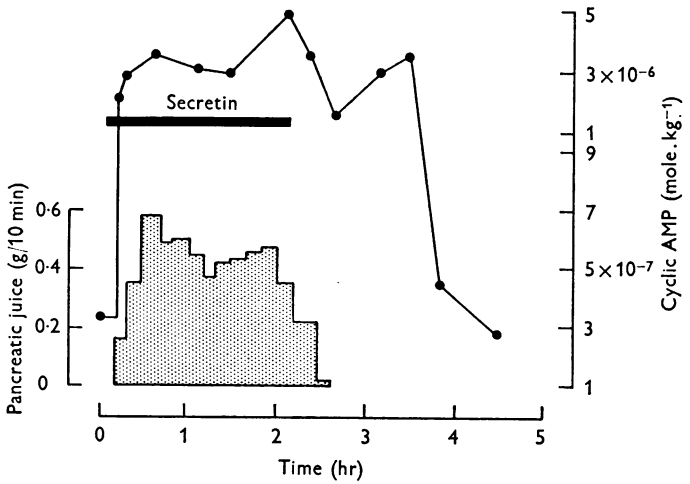

Fig. 3. Anaesthetized cat. Effect of a continuous infusion of crude (Crick–Harper–Raper) secretin (initially 20·0 then 30·0 μg.min^{-1}, I.V.), represented by the horizontal bar, on the pancreatic electrolyte secretion (stippled) and on the concentration of cyclic adenosine 3′,5′-monophosphate (●) in the pancreas, expressed as mole.kg^{-1} wet wt. at the times indicated.

during this secondary rise was approximately equal to that in the first peak. After a total of about 2 hr, cyclic AMP concentration again returned to the basal value.

A similar picture was obtained if secretin was given as a continuous infusion. In this case, however, the initial rise in cyclic AMP concentration was maintained as long as secretin was infused, after which it rose and fell secondarily as before (Fig. 3) (this can also be judged from Figs. 5, 8 and 9, which illustrate the effects of pancreozymin or acetylcholine superimposed on such a secretin infusion).

Results obtained using synthetic secretin (Fig. 6) were indistinguishable from those using crude natural secretin.

Effect of pancreozymin on cyclic AMP concentration. Pancreozymin, given as a single intravenous injection, was tested alone in five experiments, one of which is illustrated in Fig. 4. As pancreozymin stimulates only

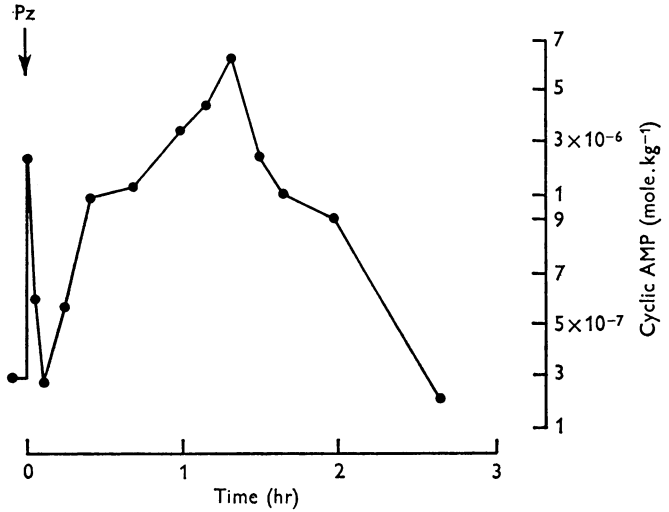

Fig. 4. Anaesthetized cat. Effect of a single injection of crude (Crick–Harper–Raper) pancreozymin (5·0 mg, I.V.), given at the arrow in the absence of secretin stimulation, on the concentration of cyclic adenosine 3′,5′-monophosphate (●) in the pancreas, expressed as mole.kg^{-1} wet wt. at the times indicated.

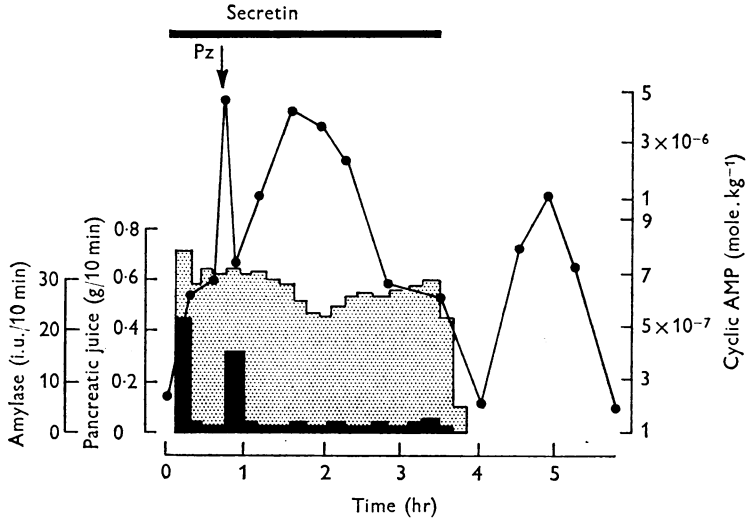

Fig. 5. Anaesthetized cat. Effect of a single injection of crude (Crick–Harper–Raper) pancreozymin (5·0 mg, I.V.), given at the arrow, on pancreatic amylase secretion (in black) and the concentration of cyclic adenosine 3′,5′-monophosphate (●) in the pancreas, expressed as mole.kg^{-1} wet wt. at the times indicated, during submaximal pancreatic electrolyte secretion (stippled) in response to an infusion of crude (Crick–Harper–Raper) secretin (20 μg.min^{-1}, I.V.) for the duration of the horizontal bar. The large, initial enzyme output (also illustrated in Figs. 6, 8 and 9) represents the washing-out of pre-formed enzyme from the duct system (for a full discussion of this phenomenon, see the previous paper: Case & Scratcherd, 1972).

enzyme secretion from the pancreas of the cat, an electrolyte secretion is required to act as a vehicle for the enzymes if the physiological response of the hormone (i.e. enzyme secretion) is to be measured simultaneously with the changes in cyclic AMP concentration. Pancreozymin was therefore also tested during a submaximal infusion of secretin (Fig. 5). In both cases, the result was the same. 30 sec after injection, the cyclic AMP concentration had risen as much as 10 times, but, within a further 30 sec, it

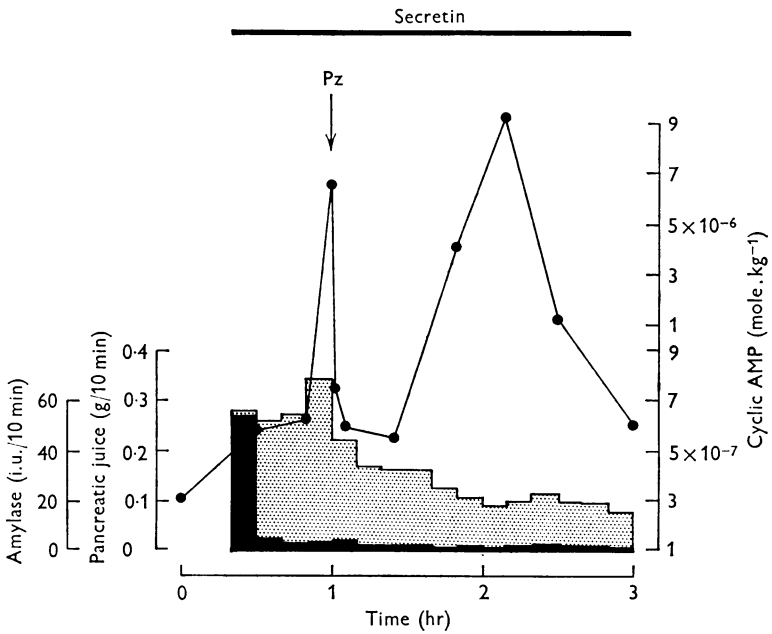

Fig. 6. Anaesthetized cat. Effect of a single injection of a very small dose of pure (Mutt) pancreozymin (0·05 μg, I.V.), given at the arrow, on pancreatic amylase secretion (in black) and the concentration of cyclic adenosine 3′,5′-monophosphate (●) in the pancreas, expressed as mole.kg^{-1} wet wt. at the times indicated, during submaximal pancreatic electrolyte secretion (stippled) in response to an infusion of synthetic (Squibb) secretin (0·04 clinical units min^{-1}, I.V.) for the duration of the horizontal bar. The initial enzyme output represents the wash-out of pre-formed enzyme from the duct system.

had reached near basal concentration again. Simultaneously, enzyme secretion was evoked. As with secretin, a large secondary rise in cyclic AMP concentration took place, dissociated from any enzyme secretion, with a time course similar to that following secretin.

Because of the impurities in the crude pancreozymin preparation pure natural cholecystokinin-pancreozymin (CCK-Pz) was also used. A very small dose of CCK-Pz (0·05 μg) did not cause a secretion of enzyme

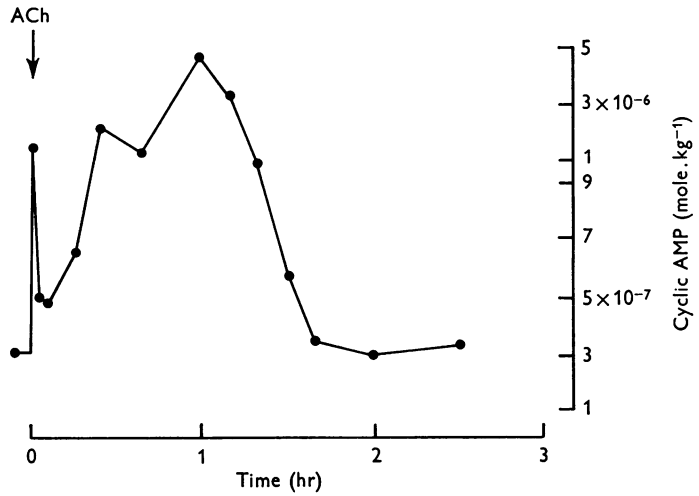

Fig. 7. Anaesthetized cat. Effect of a single injection of acetylcholine chloride (2·0 μg, I.A. above the coeliac axis), given at the arrow in the absence of secretin stimulation, on the concentration of cyclic adenosine 3′,5′-monophophate (●) in the pancreas, expressed as mole.kg^{-1} wet wt. at the times indicated.

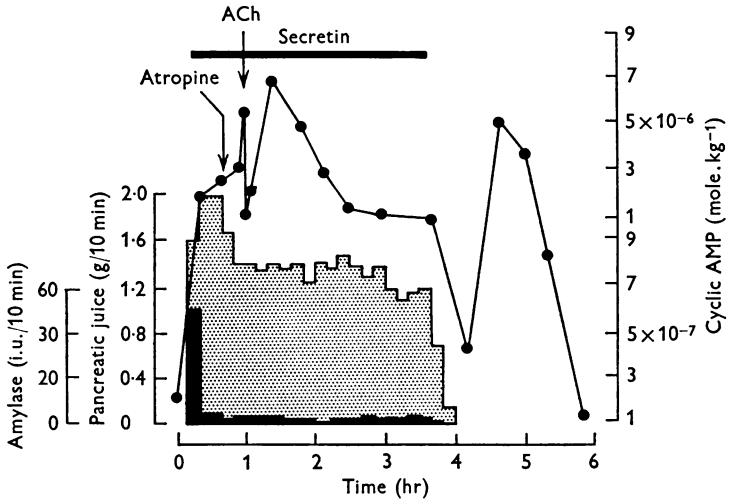

Fig. 8. Anaesthetized cat. Effect of a single injection of acetylcholine chloride (2·0 μg, I.A. above the coeliac axis), given at the second arrow on pancreatic amylase secretion (in black) and the concentration of cyclic adenosine 3′,5′-monophosphate (●) in the pancreas, expressed as mole.kg^{-1} wet wt. at the times indicated, after the prior administration of atropine sulphate (1·0 mg.kg^{-1}, I.V.) given at the first arrow. Pancreatic electrolyte secretion (stippled) was initiated and maintained by an infusion of crude (Crick–Harper–Raper) secretin (20 μg.min^{-1}, I.V.) for the duration of the horizontal bar, and resulted in the initial wash-out of pre-formed enzyme from the duct system.

although the typical pattern of cyclic AMP changes persisted (Fig. 6). At higher doses, this pure CCK-Pz preparation caused the normal secretion of enzymes.

Effect of acetylcholine on cyclic AMP concentration. Through the intermediary of acetylcholine, vagal stimulation also results in enzyme secretion from the pancreas. The effect of cholinergic stimulation on the concentration of cyclic AMP in the pancreas was therefore tested, and, to avoid the uncertainties attached to electrical stimulation of the nerve, this was

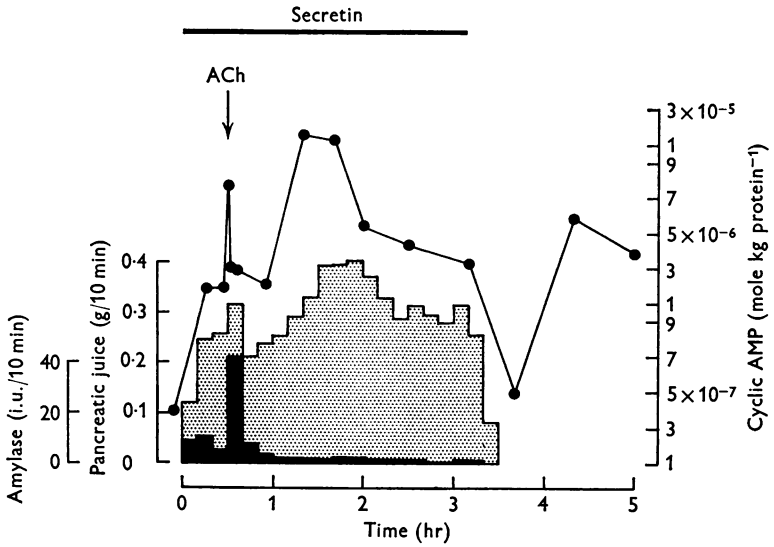

Fig. 9. Saline-perfused pancreas. Effect of a single injection of acetylcholine chloride (20 μg, added to the arterial supply close to the gland), given at the arrow, on amylase secretion (in black) and the concentration of cyclic adenosine 3′,5′-monophosphate (●) in the pancreas, expressed as mole.kg^{-1} protein at the times indicated, during submaximal electrolyte secretion (stippled) in response to an infusion of crude (Crick–Harper–Raper) secretin (20 μg.min^{-1}, added to the arterial supply) for the duration of the horizontal bar. The initial wash-out of pre-formed enzyme is spread over two periods because of the slow secretory rate.

achieved by close intra-arterial injection of 2 μg acetylcholine. The changes in cyclic AMP concentration (Fig. 7) were very similar to those obtained using pancreozymin, showing a transitory rise, followed later by a large secondary rise. On repeating the experiment after prior atropinization of the animal (2 mg atropine sulphate via the saphenous vein) the same pattern was obtained (Fig. 8), possibly slightly reduced in magnitude, while enzyme secretion was abolished.

Cyclic AMP concentration in the perfused pancreas. At an early stage in this work it seemed important to eliminate the possibility that extra-

pancreatic influences were affecting the cyclic AMP concentration, causing the large secondary rises in cyclic AMP concentration dissociated from secretion, which followed stimulation by secretin, pancreozymin and acetylcholine. This was done by using an isolated saline-perfused preparation of the pancreas. The perfusate was allowed to drain to waste after passing through the gland. Precisely the same changes in cyclic AMP concentration were observed as in the *in vivo* preparation (Fig. 9).

DISCUSSION

Theophylline, secretin, pancreozymin and acetylcholine all caused the concentration of cyclic AMP in pancreatic tissue to rise. The nature of this increase, which was often dramatic, was complex and varied according to the stimulus. The increase following a pulse of pancreozymin, which was indistinguishable from that following acetylcholine, consisted of a transitory rise lasting less than 1 min, followed by a secondary rise, of approximately equal magnitude but lasting for an hour or more. Following secretin the first rise in cyclic AMP concentration was maintained as long as the secretion evoked by this hormone was in progress. This was about half an hour if a pulse of secretin was given or longer if it was infused. If pancreozymin or acetylcholine had been infused the initial rise following these agents may also have been protracted; this point was not investigated. Although not studied in detail, theophylline also caused a rise in pancreatic cyclic AMP concentration during intravenous infusion, but the maximal concentration achieved after 20 min infusion was never as great as that observed after the humoral agents. The cyclic AMP content of isolated frog gastric mucosa was maximal only after 30–60 min exposure to theophylline (Harris, Nigon & Alonson, 1969). Pancreatic cyclic AMP concentration may continue to increase during prolonged infusion of theophylline.

In each case the effect of these agents on pancreatic cyclic AMP concentration could have been caused either by an inhibition of cyclic nucleotide phosphodiesterase, or by a stimulation of adenylate cyclase. Methyl xanthines inhibit purified phosphodiesterase preparations (Butcher & Sutherland, 1962; Nair, 1966; Cheung, 1967; Menahan, Hepp & Wieland, 1969; Kukovetz & Pöch, 1970; Ide & Okabayashi, 1970) and it is likely that in these experiments theophylline was inhibiting phosphodiesterase, which is very active in the pancreas (Butcher & Sutherland, 1962). This high phosphodiesterase activity suggests that potentially highly active adenylate cyclase is also present in the pancreas if it is assumed that the low basal cyclic AMP concentrations are determined by the equilibrium between formation and break-down (Davies, 1970). Secretin was probably

acting on adenylate cyclase for, during secretin-stimulated lipolysis in rat adipose tissue, it increases adenylate cyclase activity measured in 'ghosts' of fat cells (Rodbell, Birnbaumer & Pohl, 1970) and in cell-free homogenates of isolated fat cells (Butcher & Carlson, 1970). As yet there have been no studies on the effects of pancreozymin or acetylcholine on adenylate cyclase or phosphodiesterase. Unlike secretin, pancreozymin does not stimulate lipolysis from rat adipose tissue (Butcher & Carlson, 1970), and so presumably does not activate adenylate cyclase in that tissue. However, most hormones seem to increase cellular cyclic AMP concentrations by an action on adenylate cyclase, and at present there is no reason to suggest that pancreozymin and acetylcholine should act otherwise.

The pancreas is a heterogeneous gland, consisting of both exocrine and endocrine tissue, as well as connective tissue and the normal components of the vascular and nervous systems. Secretin, pancreozymin and probably acetylcholine all stimulate islet tissue to release insulin (Dupré, 1970) possibly through a mechanism involving cyclic AMP (see Cerasi & Luft, 1970). However, it is unlikely that this tissue, which comprises only a very small fraction of the gland (Ogilvie, 1933), could contribute significantly to the overall concentration of cyclic AMP in the gland.

The exocrine pancreas contains acinar cells and centro-acinar and duct cells. There is growing evidence that the former are the source of pancreatic enzymes, and hence are the target for pancreozymin and acetylcholine, while the latter probably secrete most, if not all, the electrolytes and water, and are therefore the target for secretin (Harper, 1967; Schulz, Yamagata & Weske, 1969). If this is so, the concentration of cyclic AMP within individual pancreatic cells may be considerably higher than analysis of the whole-gland would suggest. Furthermore, it would be expected that the increase in cyclic AMP concentration caused by secretin would be additive with that due to pancreozymin or acetylcholine. In view of the large number of variables in these experiments it is difficult to say whether this was the case; though it did not appear to be so. Perhaps secretin acts on acinar cells to increase their content of cyclic AMP.

The crucial question which must be discussed is the relation between these complex changes in cyclic AMP concentration and pancreatic secretion. The small rise in concentration following theophylline was accompanied by a sparse flow of juice. Together with earlier evidence (in which theophylline was shown to stimulate secretion in the perfused cat pancreas; Case & Scratcherd, 1972), this observation strongly suggests that a rise in cyclic AMP concentration is associated with electrolyte secretion. The initial rise in cyclic AMP concentration after secretin was also accompanied by electrolyte secretion, and it always occurred before secretion commenced, suggesting again that cyclic AMP could be involved in electrolyte

secretion. The initial rise in cyclic AMP due to pancreozymin or acetylcholine was accompanied by enzyme secretion, but not by electrolyte and water secretion. The remarkably evanescent nature of both the changes in cyclic AMP concentration, and the secretion of enzymes in response to pancreozymin (Case, Harper & Scratcherd, 1969) and vagal stimulation (Greenwell & Scratcherd, 1970), coupled with the complex architecture of the duct system, make it difficult to determine whether the rise in cyclic AMP concentration preceded secretion. However, in a drop-by-drop analysis of pancreatic secretion following pancreozymin injection, enzymes were not detected in the pancreatic juice until 45–60 sec after stimulation (Case *et al.* 1969). Therefore in view of the large rise in cyclic AMP concentration observed only 30 sec after pancreozymin or acetylcholine injection it seems likely that the cyclic AMP concentration did rise before enzyme secretion had begun.

In certain conditions the initial rise in cyclic AMP concentration was observed in the absence of enzyme secretion, namely: following a very small dose of pancreozymin, and following acetylcholine after prior administration of atropine to the animal. Furthermore, the large, consistent secondary rise in cyclic AMP concentration was never associated with secretory activity. It is therefore obvious that no simple relation exists between cyclic AMP and secretion. This is emphasized by the observation that electrolyte secretion was maximal immediately after a pulse of secretin, whereas the concentration of cyclic AMP continued to rise for at least 5 min.

These observations therefore show that while a rise in the intracellular concentration of cyclic AMP may be necessary for secretion of electrolytes and enzymes, secretion does not necessarily follow a rise in cyclic AMP concentration. Recent studies on the fat cell 'ghost' preparation of Rodbell (1967), both in his laboratory (Birnbaumer & Rodbell, 1969; Rodbell *et al.* 1970) and that of Hechter (see Hechter, 1970), point towards the existence of a number of 'signal discriminators' in the fat cell membrane, each sensitive to one of the many hormones capable of stimulating lipolysis in that cell (namely: catecholamines, ACTH, glucagon, TSH, LH and secretin). These discriminator units are all coupled to the total complement of adenylate cyclase in such a way that stimulation of any one discriminator by a hormone can cause maximal activation of the cyclase. The simple concept of a hormone receptor is therefore rapidly becoming more complex. By extending this complexity it is perhaps possible to account for the observations described here by suggesting that another part of the 'receptor system' must be activated, in addition to the discriminator associated with adenylate cyclase, before secretion can take place. It must be supposed that these other parts have a lower affinity for the hormones

than the adenylate cyclase moiety, and, in the case of acetylcholine, are inhibited by atropine. If this tentative suggestion can explain the dissociation between elevated cyclic AMP concentration and secretion during the first peak, it does not explain the second rise in cyclic AMP concentration, which was observed after secretin, pancreozymin and acetylcholine at all doses. Since it was also observed in the perfused gland, it was clearly a direct effect of these stimuli on the pancreas.

Alternatively, a messenger in addition to cyclic AMP may be necessary for secretion to occur. Two possible candidates are calcium ions and the prostaglandins. Prostaglandin action has been linked to cyclic AMP formation in a number of systems (Ramwell & Shaw, 1970) and prostaglandins E_1 and E_2 do stimulate electrolyte secretion from cat pancreas (Scratcherd & Case, 1972), probably through a mechanism involving adenylate cyclase. The interrelations between calcium ions and cyclic AMP have been described and discussed in detail by Rasmussen (1970) and the number of secretory systems in which both are involved is impressive. Certainly calcium is necessary for the secretion of amylase from pancreas *in vitro* (Hokin, 1966; Robberecht & Christophe, 1971; Case & Clausen, 1971) and of electrolytes from perfused cat pancreas (B. E. Argent, R. M. Case & T. Scratcherd, unpublished).

The time course of the second rise in cyclic AMP concentration corresponds closely to that of incorporation of [^{14}C]phenylalanine into pancreatic protein after stimulation of pigeon pancreas slices by pancreozymin (Webster, 1969). Secretin has also been claimed to be involved in enzyme secretion (Wormsley, 1969). Cyclic AMP has been implicated in protein biosynthesis in a number of ways: stimulation of amino acid uptake in liver (Chambers, Georg & Bass, 1970) and bone and kidney (Phang, Downing & Weiss, 1970); stimulation of amino acid incorporation into trichloracetic acid-insoluble proteins and amylase in rat parotid gland (Grand & Gross, 1969); depression of gene action dependent on the cyclic AMP-dependent phosphorylation of some histones (Langan, 1968); or as a factor necessary for the functioning of the ribosome (Loeb & Blat, 1970). It therefore seems possible that the secondary rise is associated with synthetic mechanisms in the gland, necessary to replenish the stores of enzymes which have been depleted during secretion, although this suggestion is not supported by the recent observations of Morisset & Webster (1971) that dibutyryl cyclic AMP administered either *in vivo* or *in vitro* is not associated with a measurable synthetic response in rat pancreas.

Although complex feed-back mechanisms, both positive and negative, could be suggested to explain the occurrence of this second rise in cyclic AMP concentration, it may be said in conclusion that the observations described here present a complex picture which seems at present to have

no simple explanation. Earlier experiments, in which the effects of dibutyryl cyclic AMP and theophylline were tested in the perfused cat pancreas, suggested that cyclic AMP was an intermediate in the action of secretin but not in that of pancreozymin (Case & Scratcherd, 1972). The observations described here would support the former suggestion (with the proviso that no simple relationship exists between cyclic AMP and electrolyte secretion), but not necessarily the latter. It may be that exogenously applied cyclic AMP is unable to mimic the large, rapid rise in intracellular cyclic AMP which is observed after pancreozymin or acetylcholine, thus accounting for this discrepancy.

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