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AMYLASE SECRETION BY THE PERFUSED CAT PANCREAS IN RELATION TO THE SECRETION OF CALCIUM AND OTHER ELECTROLYTES AND AS INFLUENCED BY THE EXTERNAL IONIC ENVIRONMENT*

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SUMMARY

1. Amylase secretion from the perfused pancreas consists of two components: a small continuous basal secretion and a stimulated secretion in response to acetylcholine or cholecystokinin-pancreozymin. The response to small doses of either stimulant was repeatable over several hours.

2. The calcium concentration of pancreatic juice, always less than that of the perfusate, was normally constant above secretory rates of 0·15 g/10 min. However, when the concentration of enzymes in the juice rose, either after stimulation or at very low secretory rates, the calcium concentration rose in parallel, suggesting that this calcium is bound to, or is a component of, pancreatic enzymes.

3. Elevation of the perfusate calcium concentration resulted in a parallel increase in the calcium concentration of the pancreatic juice.

4. Calcium-free solutions initially caused a small reduction in basal and stimulated amylase secretion and, after prolonged periods of perfusion, abolished stimulated secretion and caused a reduction in electrolyte secretion. The latter was completely reversed by calcium-rich perfusates but the effects on enzyme secretion were only partially reversible.

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5. Calcium-rich perfusates had no effect on the rate of electrolyte secretion but potentiated submaximally stimulated amylase secretion.

6. Barium did not substitute for calcium in supporting pancreatic secretion.

7. Alterations in the extracellular concentrations of sodium, potassium and magnesium had no *direct* effect on amylase secretion.

8. The local anaesthetic tetracaine inhibited amylase secretion at a lower concentration than that required to inhibit electrolyte secretion.

9. It is concluded (*a*) that calcium is secreted into the pancreatic juice in two fractions, one associated with enzymes and the other with the electrolyte component of the juice; and (*b*) that calcium ions play an important role in the stimulus-secretion coupling of pancreatic acinar cells, but that the effects of calcium depletion on electrolyte secretion may principally be due to alterations in the permeability of the duct system.

INTRODUCTION

The essential features of the synthesis, intracellular transport, and storage of digestive enzymes within the pancreatic acinar cell are well defined. After synthesis on the ribosomes of the rough-surfaced endoplasmic reticulum, the digestive enzymes, or their zymogens, are transferred via the cisternae of the rough-surfaced endoplasmic reticulum and the small vesicles of the peripheral Golgi complex to the condensing vacuoles, which are subsequently transformed into zymogen granules by progressive filling and concentration of their contents and stored in the apical region of the cell. Morphological studies have shown that, following stimulation, zymogen discharge (which we shall refer to as secretion) involves the movement of the granule to the cell surface, where its membrane fuses with the plasma membrane thus extruding its contents by exocytosis into the acinar lumen (for review see Schramm, 1967). However, little is known of the mechanical processes involved in movement of the zymogen granules, or how they are controlled (stimulus-secretion coupling).

This study explores the influence of extracellular ionic composition on these processes in the perfused cat pancreas. Such an analysis in the intact gland is complicated by the need for a background secretion of electrolytes and water (to act as a vehicle for the enzymes), which itself is markedly influenced by the composition of the perfusing fluid (Case, Harper & Scratcherd, 1968, 1969*b*). Nevertheless a perfused preparation does offer considerable advantages over alternative *in vitro* techniques, notably rapid and reversible alteration of the extracellular fluid composition, efficient oxygenation and collection of uncontaminated secretory products. In addition, the simultaneous measurement of electrolyte secretion has

allowed observations to be made on this component of the pancreatic juice.

The present observations (previously published in brief; Argent, Case, Fraser & Scratcherd, 1972) suggest that in the pancreas, as in some other secretory tissues (see, Rubin, 1970), the calcium ion plays an important role in enzyme secretion, but that other extracellular cations, magnesium, sodium and potassium, are not directly involved.

METHODS

A saline-perfused preparation of the cat's pancreas (Case *et al.* 1968) was used in all experiments. Cats of either sex, weighing 0·4–4·2 kg and denied food for 18 hr before the experiment, were anaesthetized with Nembutal (sodium pentobarbitone, 60 mg/kg I.P.) and the pancreas surgically isolated. Perfusion fluid was led from a reservoir through a heat-exchange coil and, by means of a roller pump, infused into the gland's arterial supply (the coeliac and superior mesenteric arteries) via a cannula in the aorta. The effluent from the gland was drained through the superior mesenteric vein after occlusion of the portal tract. The standard perfusion fluid isosmolal with cat's plasma had the following composition in mM: NaCl 125, KCl 4·3, $NaHCO_3$ 25, $MgCl_2$ 0·5, NaH_2PO_4 1·0, $CaCl_2$ 1·25 and glucose 5. Where perfusate potassium, magnesium or calcium concentrations were altered, isomolality was maintained by adjusting the sodium ion concentration; when sodium was removed, an osmotically equivalent amount of sucrose was added. The calcium chelator EGTA (ethyleneglycol-bis(β-aminoethyl ether)-*N*,*N'*-tetra-acetic acid) was added to calcium-free perfusates at concentrations specified in the text. The fluids were filtered through Whatman no. 2 paper before use and gassed continuously with oxygen (95%) and carbon dioxide (5%). A bank of four reservoirs allowed rapid changes in the composition of the perfusion fluids to be made.

In all experiments electrolyte secretion was stimulated by infusing secretin into the arterial cannula using a motor-driven syringe. Enzyme secretion was stimulated by rapid pulses or prolonged infusions of acetylcholine chloride or, less frequently, of cholecystokinin-pancreozymin (CCK-Pz). Secretin and CCK-Pz were prepared by the method of Crick, Harper & Raper (1949), though in some experiments purer preparations (G.I.H. Laboratory, Karolinska Institutet, Stockholm) were used.

Pancreatic juice samples were collected in plastic tubes and weighed. Either or both of two indices of enzyme secretion were measured: total protein (mg), estimated by the method of Lowry, Rosebrough, Farr & Randall (1951), and amylase activity (i.u.), estimated by the Nørby method (Lagerlöf, 1942). The concentrations of calcium and magnesium in pancreatic juice and perfusion fluids were estimated by atomic absorption spectrometry (Unicam SP 90) and sodium and potassium by flame photometry (Mark II, Evans Electroselenium Ltd). The osmolalities of the perfusion fluids were determined on the Osmet Precision Osmometer (Precision Systems Ltd).

Where statistical analysis has been employed results are expressed as the mean ± S.E.

RESULTS

Normal perfusate

Enzyme secretion by the perfused cat pancreas. There was usually no measurable secretion from the unstimulated saline-perfused cat's pancreas. In the absence of an electrolyte secretion, CCK-Pz and acetylcholine were unable to evoke any detectable amylase secretion. During secretin stimulated electrolyte secretion amylase secretion consisted of two components: a small continuous basal secretion and a stimulated secretion

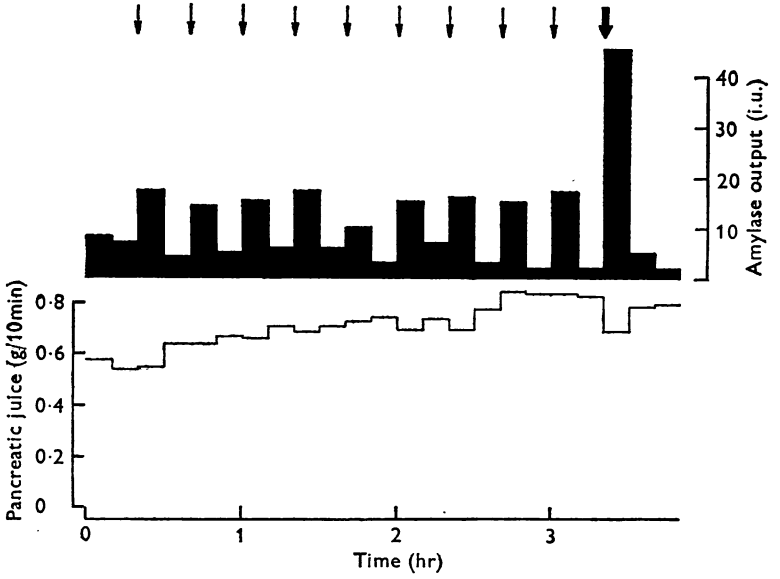

Fig. 1. The secretion of amylase by the perfused cat pancreas. Electrolyte secretion was stimulated maximally throughout by infusing crude secretin at a supramaximal dose (30 μg/min). The thin arrows represent single injections of 0·1 mg of CCK-Pz prepared by the method of Crick, Harper & Raper (1949). The thick arrow represents a larger dose (1·0 mg) of the same stimulant.

which was dose-dependent and occurred in response to exogenously administered acetylcholine or CCK-Pz. The rate of basal amylase secretion from the gland varied in different animals. Control experiments demonstrated that a fall in basal amylase secretion normally occurred during the course of an experiment (Fig. 1). If the perfused gland was stimulated to secrete large amounts of enzyme the response to the same dose of stimulant decreased during the course of an experiment. With small doses the secretory response was repeatable over several hours (Fig. 1), though the

response to the first dose of enzyme stimulant was often greater than subsequent responses, and has therefore been ignored in all experiments.

The calcium content of pancreatic juice. The concentration of calcium in pancreatic juice collected from glands stimulated maximally by secretin was always less than that of the perfusion fluid. In eleven experiments in which the gland was perfused with normal perfusate ($2{\cdot}82 \pm 0{\cdot}09$ m-equiv Ca/l.) the mean concentration of calcium in the pancreatic juice was

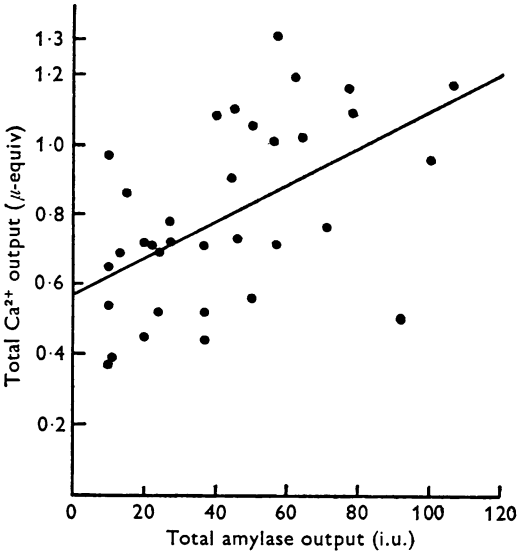

Fig. 2. The secretion of calcium and amylase iu pancreatic juice. The points represent a total of thirty-five observations from twelve experiments in which glands were stimulated to secrete amylase by various doses of acetylcholine (25 ng–1 μg). The solid line is a calculated regression line ($P = <0{\cdot}001$).

$0{\cdot}63 \pm 0{\cdot}04$ m-equiv/l. ($n = 78$). At secretory rates greater than 0·15 g/10 min the concentration of calcium was independent of flow rate. In one experiment where the gland was stimulated to secrete at flow rates below 0·15 g/10 min the concentration of calcium in the juice increased with decreasing flow rate, as did the concentration of amylase.

Following stimulation by acetylcholine the output of calcium in the pancreatic juice increased in proportion to the total amount of amylase secreted (Fig. 2). This increased output of calcium paralleled the increased output of amylase when the stimulant was administered as a pulse or as an infusion (Fig. 3).

The effects of tetracaine on pancreatic secretion. In two experiments

perfusing the isolated pancreas with solutions containing the local anaesthetic tetracaine ($2{\cdot}0$–$5{\cdot}0 \times 10^{-4}$ M) reduced the secretion of amylase in response to single doses of acetylcholine but had no effect on the volume of pancreatic secretion (Fig. 4). In three experiments concentrations of tetracaine greater than 10^{-3} M caused a reduction in the volume of pancreatic secretion.

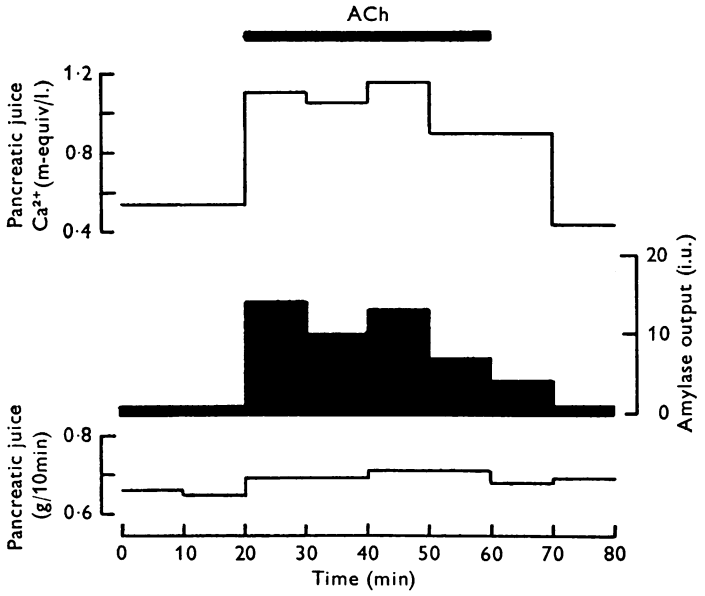

Fig. 3. Parallel output of calcium and amylase during acetylcholine infusion. Electrolyte secretion was stimulated maximally throughout by infusing crude secretin at a supramaximal dose (30 μg/min). Acetylcholine (ACh) was infused at a rate of 20 ng/min for the duration of the horizontal bar.

Alterations in perfusate [Ca] concentration

The effects of calcium-free perfusate on electrolyte and enzyme secretion. In nine experiments prolonged perfusion with calcium-free solutions containing EGTA (10^{-5} M) caused a progressive inhibition of electrolyte secretion which became apparent after 50–70 min (Fig. 5). In the early stages this inhibition could be reversed by a return to normal perfusate but when the effect had become marked, perfusion with calcium-rich fluids (6–10 m-equiv Ca/l.) was necessary for complete reversal. By increasing the EGTA concentration of calcium-free perfusates, the time required for inhibition to become apparent was reduced. With a solution containing 10^{-3} M-EGTA, the secretory rate in two experiments was reduced by a mean of 72 % after only 20 min. EGTA itself was not

responsible for the inhibition for, when added to normal (calcium-containing) perfusate, in concentrations up to 10^{-3} M, it had no inhibitory effects (two experiments).

Although reduced, basal enzyme secretion was always detectable during perfusion with calcium-free EGTA solutions, provided electrolyte secretion was maintained (Figs. 5, 6). During either the initial period of calcium-free perfusion (when electrolyte secretion was unaffected) or the early phase of

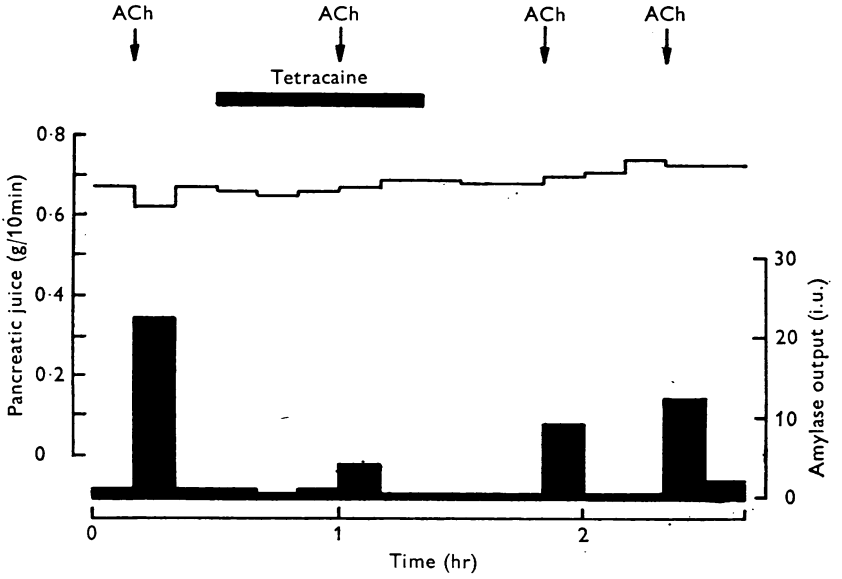

Fig. 4. The effects of the local anaesthetic tetracaine on amylase secretion. For the period denoted by the horizontal bar the fluid perfusing the gland contained tetracaine ($2{\cdot}0 \times 10^{-4}$ M). The arrows marked ACh denote single injections of 200 ng acetylcholine. Electrolyte secretion was stimulated maximally throughout by infusing crude secretin at a supramaximal dose (30 μg/min).

reduced secretory rate the response to acetylcholine was slightly diminished (Fig. 6). During the latter stages of reduced electrolyte secretion the response to acetylcholine was virtually abolished (Fig. 5), and, unlike electrolyte secretion, it was not fully restored by perfusion with calcium-rich fluids.

On returning to normal perfusate after prolonged calcium-free perfusion the concentration of calcium in pancreatic juice was elevated when compared to the control period and often equal to the concentration in the perfusing fluid (Fig. 5).

Calcium-free solutions had no effect on the rate of perfusion through the gland. In this series of experiments, similar results were obtained when

CCK-Pz and pure secretin were substituted for acetylcholine and crude secretin respectively.

The effects of calcium-rich perfusates on pancreatic secretion. Perfusion with fluids containing 10 m-equiv Ca/l. did not affect the rate of pancreatic secretion stimulated maximally or submaximally by secretin, but did cause the juice calcium concentration to rise and remain elevated throughout the 30 min test period (Fig. 7). In three of seven such experiments a small increase (mean 120 %) in basal amylase secretion was also observed on switching to calcium-rich buffer, but the effect was transient, being observed only in the first 10 min period of perfusion.

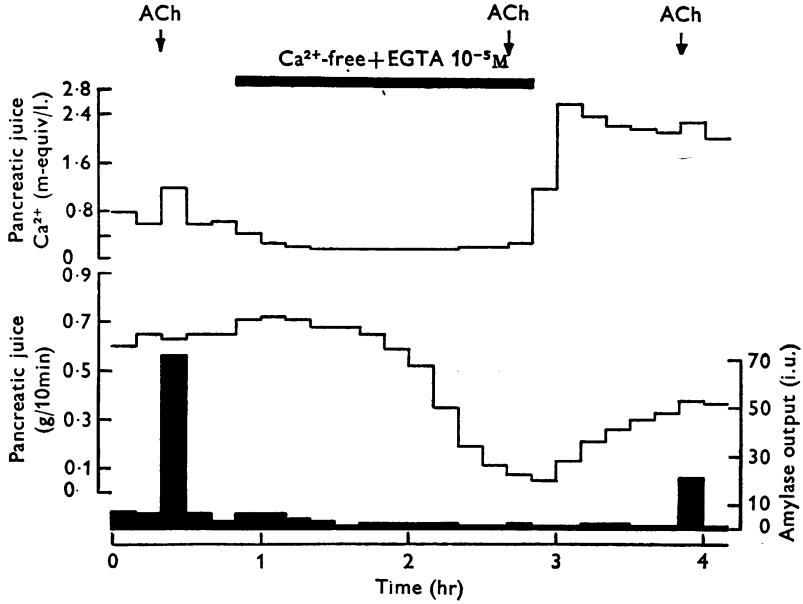

Fig. 5. The effect of prolonged perfusion with a calcium-free solution on electrolyte and amylase secretion from a perfused cat pancreas. Electrolyte secretion was stimulated maximally throughout by infusing crude secretin at a supramaximal dose (30 μg/min). A Ca-free solution containing EGTA (10^{-5} M) was perfused through the gland for the duration of the horizontal bar. The arrows marked ACh indicate single injections of acetylcholine (ACh, 200 ng).

In three further experiments, increasing the calcium concentration of the perfusate during minimal acetylcholine infusion (5 ng/min) caused a large increase in amylase secretion (Fig. 8). This effect was not wholly due to the release of acetylcholine from nerve terminals within the gland, since it was also observed during stimulation by CCK-Pz ($2{\cdot}5 \times 10^{-2}$ Crick-Harper-Raper u./min) in the presence of atropine (10 mg/l.) in three experiments.

The effects of barium on pancreatic secretion. In two experiments barium (2·5–5·0 m-equiv/l.) did not prevent the reduction in electrolyte and amylase secretion associated with prolonged calcium-free perfusion or aid the recovery of electrolyte secretion after calcium depletion.

Alterations in perfusate [Mg]

The effect of magnesium-rich perfusate on pancreatic secretion. Perfusion with magnesium-rich fluids (10 m-equiv Mg/l.) for up to 60 min did not inhibit electrolyte secretion or acetylcholine-stimulated amylase secretion.

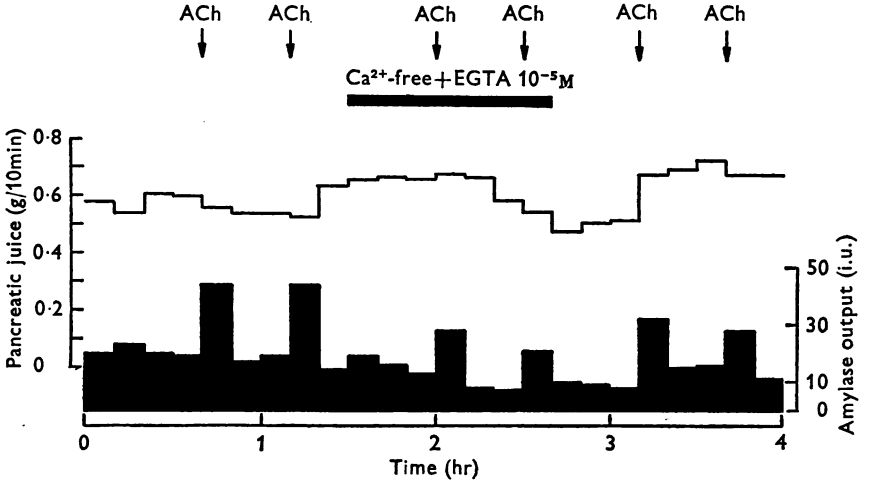

Fig. 6. The effect of calcium-free perfusate on amylase secretion from a perfused cat pancreas. Electrolyte secretion was maximally stimulated throughout by infusing secretin at a supramaximal dose (30 μg/min). A calcium-free solution containing EGTA (10^{-5} M) was perfused through the gland for the duration of the horizontal bar. The arrows marked ACh indicate single injections of acetylcholine (200 ng).

In fact, like calcium, excess magnesium caused a transient increase in basal enzyme secretion and potentiated minimal stimulation by acetylcholine, but the effects were never as great as those observed with calcium.

The effects of magnesium-free perfusates on pancreatic secretion. Magnesium-free solutions had no effect on electrolyte secretion or on acetylcholine-stimulated enzyme secretion when perfused through the gland for up to 60 min (two experiments).

Alterations in perfusate [Na]

The effect of sodium-deficient perfusates on pancreatic secretion. Sodium deficiency is known to inhibit pancreatic electrolyte secretion (Case *et al.* 1968). Its effect on enzyme secretion was tested in five experiments by perfusion with solutions containing either 0 or 50 mM-Na/l. (Fig. 9). After 50 min perfusion with 50 mM-Na/l., the response to acetylcholine was

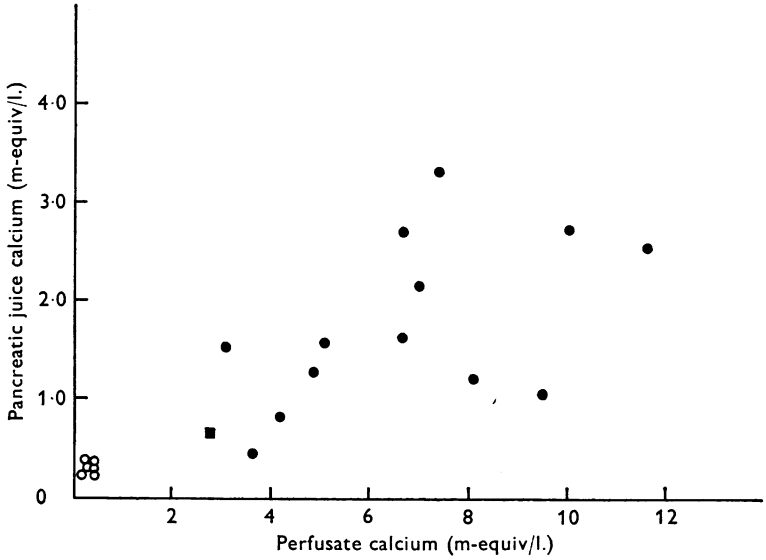

Fig. 7. The relationship between the concentration of calcium in perfusate and pancreatic juice. The closed circles represent single observations from a total of three experiments in which the concentration of calcium in the perfusion fluid was varied. The open circles represent single observations from six experiments in which the gland was perfused with a calcium-free solution containing EGTA. The filled square represents seventy-eight observations from eleven experiments in which the gland was perfused with the normal perfusion fluid.

normal though, because of the slow secretory rate, the enzyme was not all eliminated from the duct system during the 10 min test period, most of it appearing in the period following return to normal perfusate. A return to normal perfusate alone produced only a minimal increase in enzyme secretion, which presumably was a washing out of basal secretion that had accumulated in the ducts during the period of low electrolyte secretion. Similar observations were obtained with sodium-free solutions, though in these experiments electrolyte secretion often ceased completely.

Alterations in perfusate [K]

The effect of potassium-free solutions on pancreatic secretion. Potassium omission reduces pancreatic electrolyte secretion by about 60 % (Case, Harper & Scratcherd, 1969*b*). However, in three experiments, potassium-free fluids did not affect acetylcholine-stimulated amylase secretion (Fig. 10). In these experiments an increase in basal enzyme secretion was observed during potassium omission, an effect which was blocked by atropine.

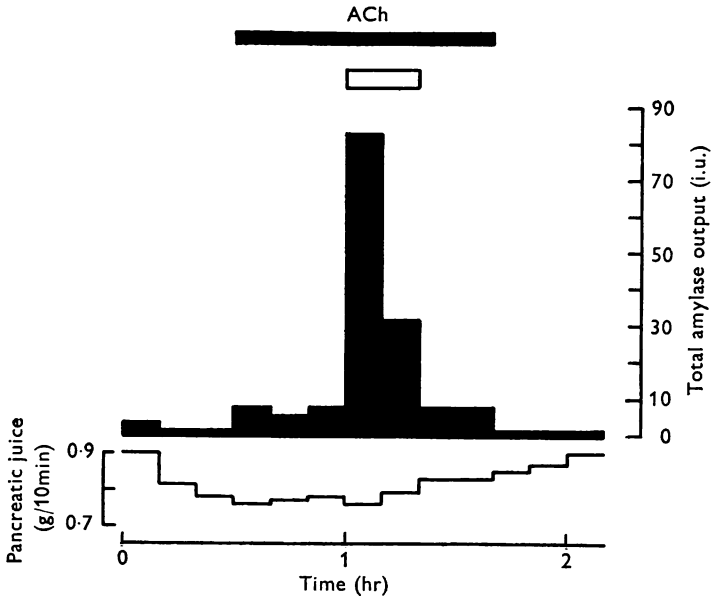

Fig. 8. The effect of increasing the perfusate calcium concentration during submaximal amylase secretion. Acetylcholine (5 ng/min) was infused into the gland for the period indicated by the filled bar. For the duration of the open bar the perfusate contained calcium at a concentration of 10 m-equiv/l. Electrolyte secretion was maximally stimulated throughout by infusing crude secretin at a supramaximal dose (30 μg/min).

The effect of potassium-rich perfusates on pancreatic secretion. Solutions containing potassium at concentrations greater than 30 mM/l. cause acetylcholine release from nerve terminals within the pancreas and thus secondarily cause amylase secretion (Argent, Case & Scratcherd, 1971). This observation alone suggests that the enzyme secretory process of the acinar cell is unaffected by high extracellular potassium concentrations. In seeking support for this view, the effect of potassium-rich perfusion fluid on CCK-Pz-stimulated secretion was tested in three experiments. The

perfusion fluid contained atropine sulphate (10 mg/l.) to block the action of released acetylcholine. The response to CCK-Pz was unaffected by high extracellular potassium concentrations (Fig. 11).

DISCUSSION

Amylase secretion by the isolated, perfused cat pancreas is clearly very similar to that described previously for the gland *in vivo* (Case, Harper & Scratcherd, 1969*a*), consisting of a small, continuous basal secretion which

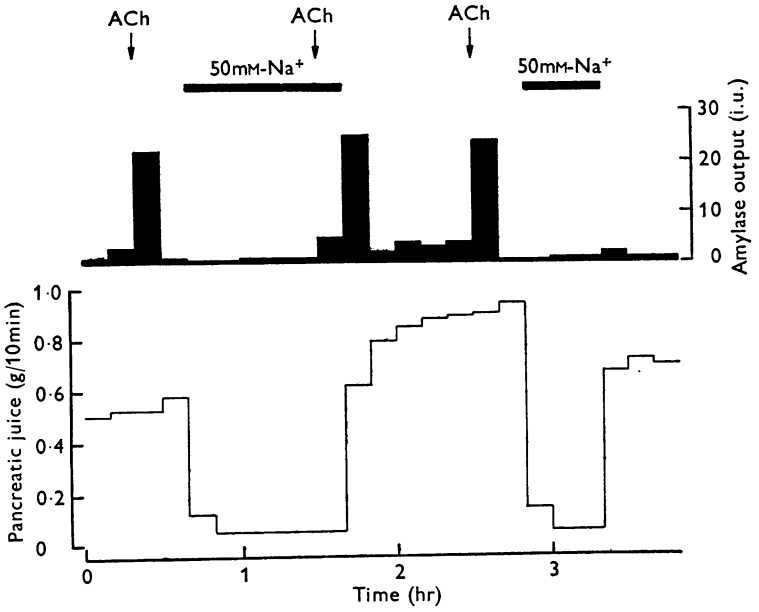

Fig. 9. The effects of sodium deficiency on electrolyte and amylase secretion from the perfused cat pancreas. Electrolyte secretion was stimulated maximally throughout by infusing crude secretin at a supramaximal dose (30 μg/min). For the duration of the horizontal bars the gland was perfused with a solution containing 50 mM-Na/l., isotonicity being maintained with sucrose. The arrows marked ACh indicate single injections of acetylcholine (200 ng).

is supplemented in response to acetylcholine or CCK-Pz. As the perfusion fluids did not contain amino acids, the ability of the isolated gland to synthesize enzymes is limited by the size of the intracellular pool of amino acids, which accounts for the decreasing enzyme output in response to repeated large doses of enzyme stimulant. However, the consistent response to repeated small doses validates the use of this preparation in studying enzyme secretion.

The secretion of calcium in pancreatic juice. During maximal stimulation with secretin alone, the juice calcium concentration was about one quarter of that in the perfusate. Whenever the concentration of amylase in the secretion rose, either because the electrolyte secretory rate was very slow, or during transient or maintained stimulation with acetylcholine or CCK-Pz, the concentration of calcium rose in parallel. These observations confirm those made previously in the intact dog (Zimmerman, Dreilling, Rosenberg

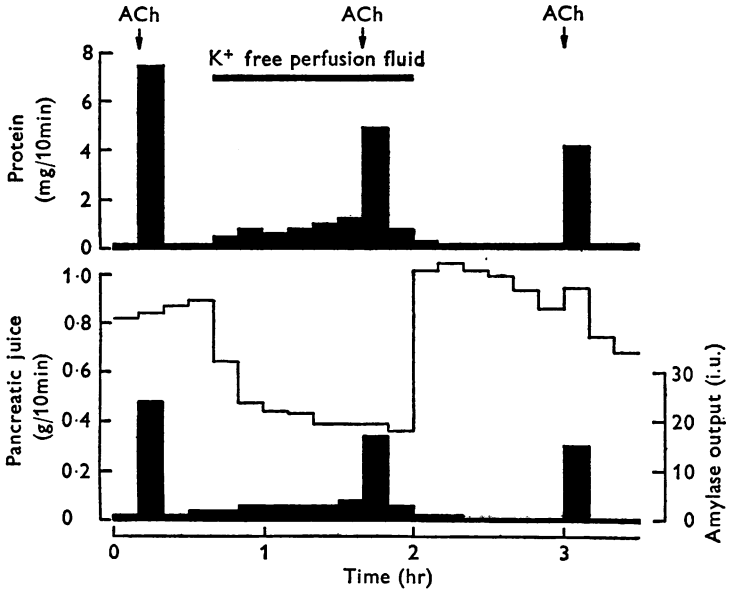

Fig. 10. The effects of potassium-free perfusion fluid on amylase, protein and electrolyte secretion from a perfused cat pancreas. Electrolyte secretion was maximally stimulated throughout by infusing crude secretin at a supramaximal dose (30 μg/min). For the period denoted by the horizontal bar potassium was absent from the perfusion fluid. The arrows marked ACh indicate single injections of acetylcholine (200 ng).

& Janowitz, 1967; Zimmerman, Moore, Dreilling & Janowitz, 1971; Goebell, Steffan & Bode, 1972) and in man (Goebell, Bode & Horn, 1970). A similar close parallelism in the concentration of calcium and exportable protein exists in gastric (Moore & Makhlouf, 1968) and salivary (Dreisbach, 1967; Wallach & Schramm, 1971) secretions. Wallach & Schramm (1971) suggest that, in the parotid gland, calcium is packaged along with the exportable protein (calcium is known to form an internal chelate within the amylase molecule; Stein, Hsiu & Fischer, 1964; Hsiu, Fischer & Stein, 1964) and calcium may be similarly packaged in the pancreas, as zinc (Pekas, 1971) and inorganic sulphate (Berg & Young, 1971) are known to be.

However, not all calcium enters pancreatic juice bound to enzymes. Use of the saline-perfused pancreas allows the perfusate calcium concentration to be raised without the complication of binding to plasma proteins. Under such conditions the juice calcium concentration rose without a parallel increase in amylase secretion. Also, extrapolation of the regression line correlating the calcium and amylase outputs (Fig. 3) indicates that at zero

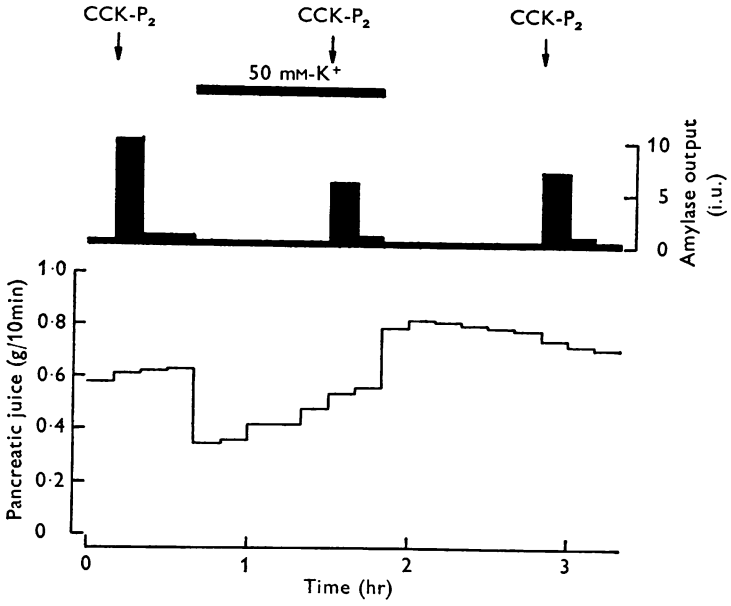

Fig. 11. The effect of excess potassium on amylase secretion from the perfused cat pancreas. For the duration of the horizontal bar the gland was perfused with a solution containing 50 mM-K. The arrows indicate single injections of pure CCK-Pz (1·0 Crick, Harper, Raper u.). To prevent the action of acetylcholine released by potassium from nerve terminal within the gland all perfusion fluids contained atropine (10 mg/l.). Electrolyte secretion was maximally stimulated throughout by infusing crude secretin at a supramaximal dose (30 μg/min).

amylase output pancreatic juice still contains calcium. This second calcium component must arise either by way of the electrolyte secretory mechanism or by diffusion through the duct system. A similar two-component hypothesis for pancreatic calcium secretion has been formulated independently by Goebell *et al.* (1972).

Effect of calcium and other cations on enzyme secretion. Potassium-rich fluids cause acetylcholine release from nerve terminals within the pancreas (Argent *et al.* 1971). The present experiments suggest that potassium-free fluids act in a similar way. Both procedures cause a secondary stimulation

of amylase secretion, due to the acetylcholine released, but do not directly stimulate the acinar cell to secrete enzymes, or influence the stimulatory effect of CCK-Pz or acetylcholine on these cells. Therefore the small depolarization of acinar cells due to acetylcholine and CCK-Pz (Dean & Matthews, 1972; Petersen & Matthews, 1972; J. R. Greenwell and T. Scratcherd, in preparation), although reflecting an altered membrane permeability, is presumably not responsible for enzyme secretion *per se*. Similar conclusions have been reached with regard to the actions of acetylcholine on the adrenal medulla (Douglas & Rubin, 1963; Douglas, Kanno & Sampson, 1967) and ACTH on the adrenal cortex (Matthews & Saffran, 1967; Jaanus, Rosenstein & Rubin, 1970).

Although the most likely cause of this small depolarization is an influx of external cation, removal of both major external cations (sodium and calcium) had little or no immediate effect on the secretory response to acetylcholine or CCK-Pz. Ridderstap & Bonting (1969) have also shown that basal enzyme secretion from isolated rabbit pancreas is unaffected during prolonged exposure to bathing fluid containing 25 m-mole Na/l. This point requires further clarification.

Although calcium removal had little immediate effect on amylase secretion, prolonged perfusion with calcium-free EGTA buffer did reduce basal secretion and almost abolish stimulated secretion. Inhibitory effects of calcium-free media on amylase secretion have been observed in other *in vitro* preparations of pancreas (Hokin, 1966; Robberecht & Christophe, 1971; Case & Clausen, 1971*a*, *b*) and parotid gland (Rasmussen & Tenenhouse, 1968; Selinger & Naim, 1970). However, inhibition was only partially reversible, suggesting that it may result from damage to the acinar cell.

In seeking evidence for a more direct role of extracellular calcium in enzyme secretion, the effects of tetracaine and of elevated extracellular calcium and magnesium concentrations were tested. The local anaesthetic tetracaine is known to inhibit catecholamine secretion from chromaffin cells by blocking the influx of calcium ions that occurs in response to acetylcholine stimulation (Douglas & Kanno, 1967). It certainly does inhibit amylase secretion at a concentration which is without effect on electrolyte secretion. However, tetracaine is also antagonistic to the action of acetylcholine (Rubin, Feinstein, Jaanus & Paimre, 1967), and this may explain its effect on the pancreas. The potentiating effects of excess calcium were rather small, and the inhibitory effects of excess magnesium non-existent; only weak inhibitory effects of magnesium have previously been described (Robberecht & Christophe, 1971) and there are no other reports of the effects of excess calcium.

The conclusion from this evidence is that extracellular calcium may not

play a very important direct role in enzyme secretion from the exocrine pancreas. This is in marked contrast to its established role in the secretion of transmitter from nerve endings and of hormones from endocrine cells where secretion appears to be triggered by calcium influx into the cell. In these tissues, secretion is directly related to extracellular calcium concentration and magnesium acts as an antagonist and barium as an agonist of calcium (see Rubin, 1970). Douglas (1968) has suggested that, in the secretion of macromolecules, calcium ions act as a coupling agent between the stimulus and the secretory mechanism. If this is the case in the pancreas the evidence above suggests that the source of the calcium ions may not be extracellular (as in neuro-endocrine tissue) but intracellular. This conclusion is supported by ^{45}Ca flux studies in rat pancreas, where acetylcholine and CCK-Pz have no effect on ^{45}Ca uptake, but do cause a dose-dependent acceleration of ^{45}Ca efflux (Case & Clausen, 1971*a*, *b*; R. M. Case and T. Clausen, in preparation). However, the rat pancreas responds differently to gastrointestinal hormones than the cat pancreas (Dockray, 1972) and it may be unwise to compare too closely data obtained in the two species. Intracellular calcium may also be used in the regulation of amylase secretion from salivary glands (Nielsen & Petersen, 1972). A more detailed consideration of these points is presented elsewhere (Case, 1973).

Effect of calcium on electrolyte secretion. The inhibitory effect of calcium-free media on pancreatic electrolyte secretion, which has not previously been described, was slower to develop than that on enzyme secretion. A similar situation exists in the submaxillary gland (Douglas & Poisner, 1963). This suggests a different sensitivity of the two secretory processes to calcium. This delayed inhibitory effect is in marked contrast to the immediate effects produced by removal of sodium, potassium or bicarbonate in pancreas (Case *et al.* 1968, 1969*b*; Case, Scratcherd & Wynne, 1970) or of sodium in the submaxillary gland (Martinez & Petersen, 1972).

A calcium requirement for gastric acid secretion has also been demonstrated (Forte & Nauss, 1963; Jacobson, Schwartz & Rehm, 1965). The cause of inhibition in these and other electrolyte transport processes is difficult to assess. It may result from alterations in the permeability of the cell membrane (Manery, 1966); or of the junctional complex between cells, which apparently acts as the principal route of passive ion permeation in gall bladder, and perhaps other tissues (Diamond, Barry & Wright, 1971). Certainly EDTA treatment causes increased movement of sucrose across bullfrog gastric mucosa (Forte & Nauss, 1963) by loosening the junctional complex between gastric cells (Sedar & Forte, 1964) and a similar explanation may account for the increased calcium concentration in pancreatic juice collected immediately after a prolonged period of calcium-free

perfusion. Whether calcium ions have a direct effect on the electrolyte secretory mechanism remains to be determined.

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