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Control of pancreatic amylase release *in vitro*: effects of ions, cyclic AMP, and colchicine

L. BENZ, B. ECKSTEIN, E. K. MATTHEWS AND J. A. WILLIAMS*

Department of Pharmacology, University of Cambridge, Cambridge, England

Summary

1. The time course and concentration-response relationship of amylase release from pieces of guinea-pig pancreas *in vitro* in response to bethanechol and pancreozymin was determined.

2. Removal of Ca^{++} from the medium had no effect on basal amylase release but abolished the stimulating effect on release of bethanechol.

3. Elevation of the concentration of Mg^{++} in the medium increased basal amylase release and reduced the response to bethanechol.

4. Elevation of the concentration of K^{+} in the medium increased amylase release; this effect was blocked by a concentration of atropine which blocked also the response to bethanechol.

5. Cyclic AMP, dibutyryl cyclic AMP and theophylline failed to stimulate amylase release. Pancreatic cyclic AMP concentrations were found not to be increased by bethanechol, pancreozymin or an elevated concentration of K^{+} in the medium.

6. Colchicine had no effect on basal amylase release or the response to bethanechol or pancreozymin.

7. It is concluded that the coupling of stimulus to secretion involves ionic control but that neither cyclic AMP production nor microtubular mechanisms play a major role in controlling exocytosis in the pancreatic acinar cell. These findings are discussed in relation to the stimulus-secretion coupling processes in other cells.

Introduction

The exocrine part of the pancreas has been a fruitful system for studying the synthesis and secretion of exportable protein. The studies of Palade have established the mechanism and kinetics of the process by which pancreatic enzymes are packaged into secretory granules and the contents extruded from the cell by exocytosis (Palade, 1959; Jamieson & Palade, 1967a, b). Control of enzyme release from the pancreatic acinar cell, however, has not been studied in as great a detail as release processes in other secretory cells, such as the adrenal medulla. Douglas and his colleagues have suggested a general mechanism for control of exocytosis which they have termed stimulus-secretion coupling (Douglas, 1968); in this scheme the stimulant molecule combines with a membrane receptor leading to membrane depolarization and an influx of Ca^{++}, which, in an as yet unknown way, promotes exocytosis. That this model might apply to pancreatic amylase

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release is suggested by the finding that pancreozymin and cholinergic agents cause depolarization of the acinar cells (Dean & Matthews, 1972) and that release is reduced in Ca^{++}-free media (Hokin, 1966; Robberecht & Christophe, 1971). Adenosine 3′,5′-cyclic monophosphate (cyclic AMP) has also been implicated in the control of pancreatic enzyme release (Kulka & Sternlicht, 1968; Ridderstap & Bonting, 1969; Morisset & Webster, 1971) but its importance in this respect has recently been questioned (Bauduin, Rochus, Vincent & Dumont, 1971). In some systems microtubules have been implicated in exocytosis (Lacy, Howell, Young & Fink, 1968; Poisner & Bernstein, 1971) but this does not appear to apply in all cases (Temple, Williams, Wilber & Wolff, 1972).

We have looked at the relative importance of these various factors in the control of amylase release from pieces of guinea-pig pancreas stimulated by carbamyl-β-methylcholine (bethanechol) and pancreozymin. An absolute dependence on Ca^{++} was found with no evidence in support of a primary role for cyclic AMP or the involvement of a microtubular mechanism.

Methods

All studies were carried out with mature albino guinea-pigs of either sex, fasted 20–24 h prior to use. The animals were stunned by a blow to the head, bled from the neck, and the pancreas quickly removed and trimmed of all mesentery, fat, and blood vessels. The pancreas was cut with scissors into pieces of 20–30 mg which were then pooled, weighed on a torsion balance and preincubated in Krebs-Henseleit bicarbonate (KHB) solution (Krebs & Henseleit, 1932). The solution had the following composition: NaCl 118 mM, KCl 4·7 mM, $CaCl_2$ 2·56 mM, $MgCl_2$ 1·13 mM, $NaHCO_3$ 25 mM, NaH_2PO_4 1·15 mM, glucose 5·6 mM. When the ionic composition was altered, the concentration of NaCl was changed to maintain isotonicity. All incubations were carried out in polythene flasks containing 3 ml of KHB at 37° C with 95% O_2–5% CO_2 as the gas phase. The flasks were shaken at 50 cycles/min. For amylase determinations 3 or 4 pieces of pancreas, total weight approximately 100 mg, were used for each flask whereas only 1 or 2 pieces per flask were used for cyclic AMP determinations. Unless otherwise noted, the pancreatic tissue was preincubated for 15 min in KHB and then incubated for 1 h in flasks containing fresh KHB and specified additions. Following incubation, the pancreas and medium were separated and the appropriate assay was carried out.

Amylase released into the medium was assayed according to the method of Rinderknecht, Wilding & Haverback (1967). Each assay tube contained 20 mg amylose azure-blue, 0·4 mmol $NaHPO_4$ pH 6·9, 0·1 mmol NaCl and 1 ml of the KHB incubation medium in a total volume of 4 ml. For determination of total pancreatic amylase, tissue was homogenized in 0·03 M phosphate buffer, pH 6·9, and a suitable aliquot added in place of the KHB medium. After incubation for 30 min at 37° with continuous shaking, the assay mixture was filtered through Whatman No. 1 paper and the absorbance of the filtrate at 595 nm determined. The amylase concentration was determined from a standard curve obtained with crystalline α-amylase and expressed as (μg amylase/mg pancreas)/hour.

Pancreatic cyclic AMP determinations were carried out by the method of Gilman (1970) after homogenization in 1 ml of 5% trichloroacetic acid followed by ether extraction. ^{3}H-cyclic AMP (Schwarz Bioresearch, 16 Ci/mmol) was used

at a concentration of 10 nM with non-radioactive cyclic AMP added to give a standard curve for 0·2–15 pmol cyclic AMP/tube.

The source of other compounds was as follows: carbamyl-β-methylcholine (bethanechol), Koch–Light Ltd.; atropine and theophylline, B.D.H.; colchicine, adenosine 3′,5′-cyclic monophosphate (cyclic AMP) and crystalline α-amylase, Sigma; N^6-2′0-dibutyryl adenosine 3′5′-cyclic monophosphate (dibutyryl cyclic AMP), Boehringer-Mannheim; amylose azure-blue, Cal Biochem. Pancreozymin and secretin were kindly provided by the GIH Research Unit, Chemistry Department, Karolinska Institutet, Stockholm, Sweden. Concentrations of pancreozymin are given in Crick, Harper and Raper units whereas secretin concentrations are expressed in Clinical units. All other reagents used were the best grade commercially available.

Student's *t* test was used for statistical analysis.

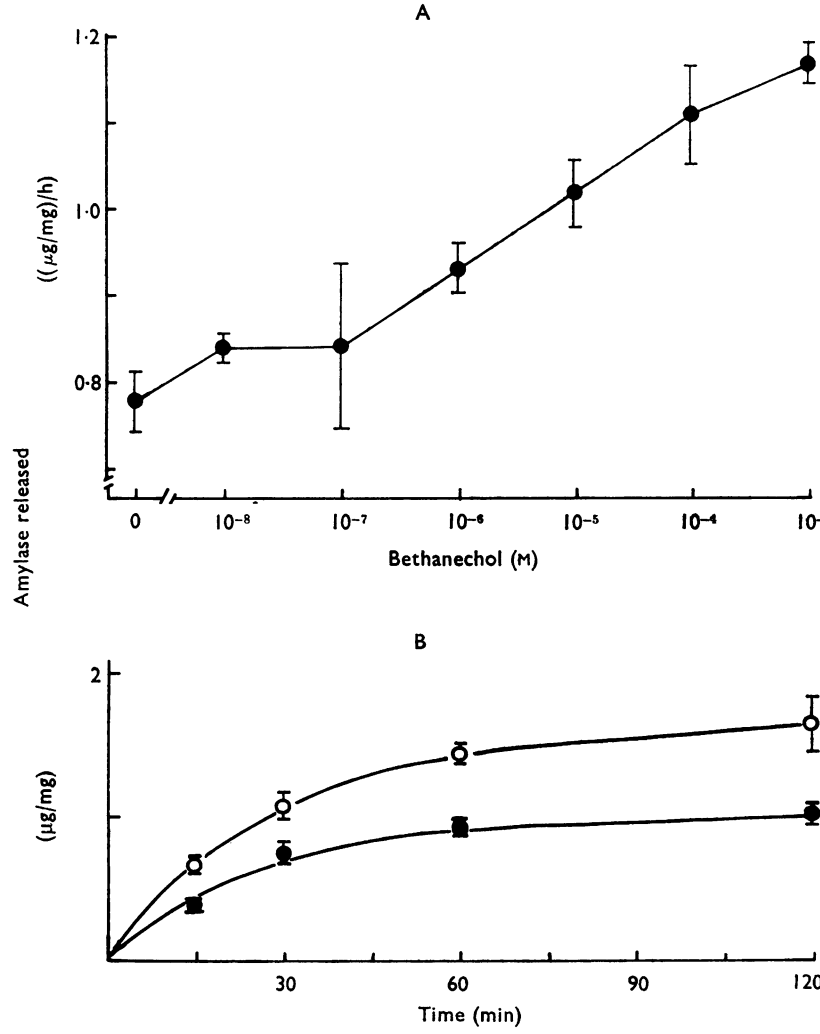

FIG. 1. Effect of bethanechol on amylase release from pieces of guinea-pig pancreas *in vitro*. (A) Amylase released in 1 h as a function of the concentration of bethanechol. (B) Amylase released as a function of time; (●——●), control; (○——○), 10^{-4}M bethanechol. All points are the mean±S.E. of 4 to 10 observations.

Results

Amylase release from guinea-pig pancreas in vitro

The effect of bethanechol and pancreozymin on the release of amylase from pieces of guinea-pig pancreas is shown in Figs. 1 and 2. Stimulation of amylase release was statistically significant ($P<0{\cdot}05$) for bethanechol at concentrations of 10^{-6}M and above, and for pancreozymin at 100 mU/ml and above. This requirement of high concentrations of pancreozymin is probably a species phenomenon since similar studies, with mouse pancreas, showed a maximal response at 30 mU/ml. Additions of soyabean trypsin inhibitor or trasylol to the medium had

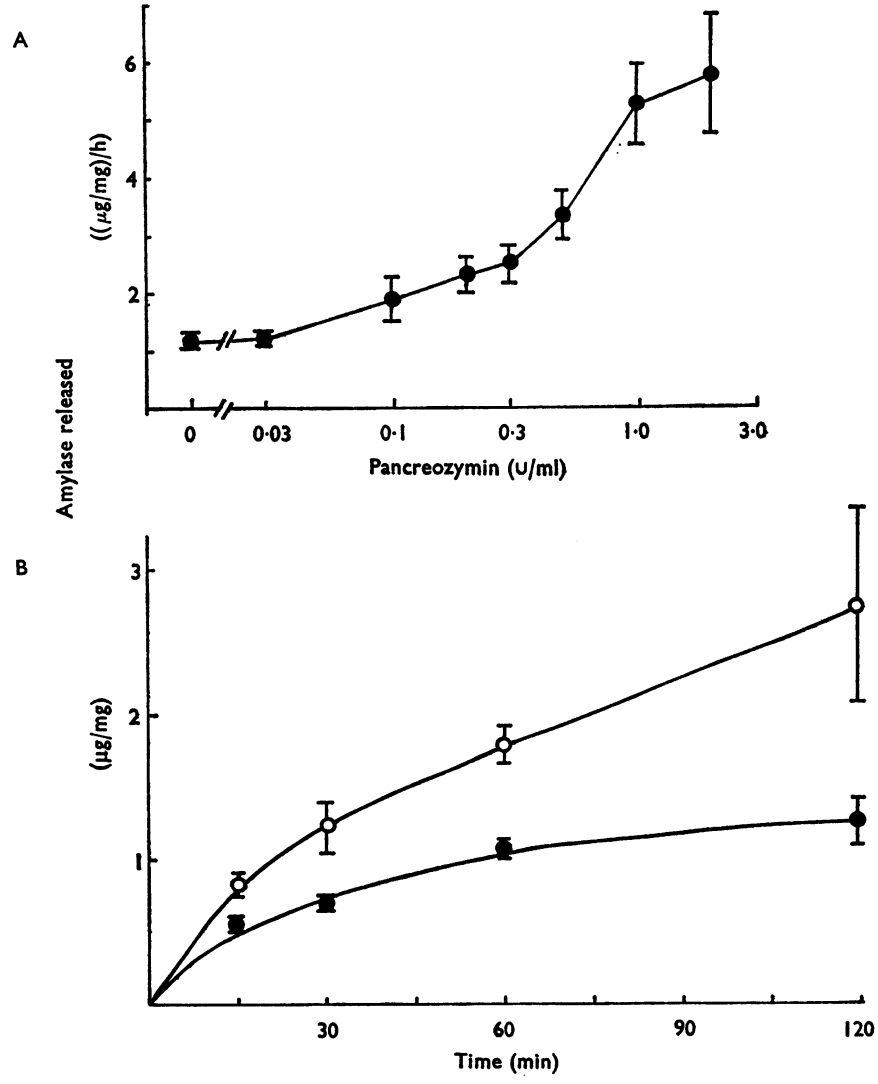

FIG. 2. Effect of pancreozymin on amylase release from pieces of guinea-pig pancreas *in vitro*. (A) Amylase released in 1 h as a function of the concentration of pancreozymin (Crick, Harper, Raper units). (B) Amylase released as a function of time: (●——●), control; (○——○), 500 mU/ml pancreozymin. All points are the mean ± S.E. of 6 to 10 observations.

no effect on the response of pieces of the guinea-pig pancreas to pancreozymin. The maximal response to pancreozymin, however, was much greater than that seen with bethanechol. The time course of the response to both agents was similar, with a continuous release of enzyme. Both basal and stimulated amylase release was greatest during the initial 15 minutes. The pancreozymin stimulation, however, was better maintained than the bethanechol stimulation and this accounts for a greater release at longer times of incubation.

In a separate experiment in which total pancreatic amylase content was also determined, 8% of the total pancreatic amylase was released during 15 min preincubation and 8·1% during a subsequent 1 h control incubation. When 10^{-4}M bethanechol was present during the 1 h incubation, amylase release was increased to 15·0%.

The comparatively large and variable basal output of amylase relative to the increment produced on stimulation observed in these experiments has been previously noted in pancreatic tissue *in vitro* for amylase (Hokin & Hokin, 1962; Kulka & Sternlicht, 1968) and other pancreatic enzymes (Bauduin, Colin & Dumont, 1969).

Effects of calcium and magnesium

The results of reducing the calcium concentration in the medium on the basal and the bethanechol-stimulated release of amylase are shown in Table 1. While the removal of Ca^{++} had no effect on basal release, reduction in the Ca^{++} concentration from 2·6 mM to 1·3 mM reduced the bethanechol-stimulated release and removal of Ca^{++} essentially abolished bethanechol stimulation.

Since Ca^{++}-dependent systems are frequently inhibited by high concentrations of Mg^{++} this was studied and the results are given in Table 2. Increasing the concentration of Mg^{++} in the medium increased the basal release, but no clear

TABLE 1. *Effect of reducing the concentration of Ca^{++} in the medium on basal and bethanechol-stimulated amylase release from guinea-pig pancreas* in vitro

Medium	Amylase release ((μg/mg)/h) Basal	10^{-4} M Bethanechol
KHB	0·68±0·04	0·95±0·05
×0·5 Ca^{++}	0·64±0·05	0·84±0·04*
0 Ca^{++}	0·66±0·04	0·70±0·04**

All values are the mean±S.E. of 10 determinations on tissue from 5 animals. The normal Ca concentration is 2·56 mM. * $P<0·05$ as compared to response in KHB. ** $P<0·01$ as compared to response in KHB.

TABLE 2. *Effect of increasing the concentration of Mg^{++} in the medium on basal and bethanechol-stimulated amylase release from guinea-pig pancreas* in vitro

Medium	Amylase release ((μg/mg)/h) Basal	10^{-4} M Bethanechol
KHB	0·65±0·01 (13)	1·10±0·03 (13)
×5 Mg^{++}	0·77±0·04 (15)**	0·94±0·04 (15)**
KHB	0·72±0·04 (13)	1·16±0·04 (13)
×10 Mg^{++}	0·90±0·06 (13)*	1·00±0·05 (13)*
KHB	0·58±0·01 (7)	0·99±0·10 (7)
×20 Mg^{++}	0·70±0·05 (7)**	0·96±0·05 (7)

All values are the mean±S.E. of the number of determinations shown in parentheses on tissue from 5 animals. The normal Mg^{++} concentration is 1·13 mM. * $P<0·05$ as compared to response in KHB. ** $P<0·01$ as compared to response in KHB.

concentration-dependence was found. By contrast, the release in the presence of bethanechol was slightly decreased. As a result, the bethanechol-stimulated increment in amylase release, over and above the basal release, was reduced by 50–85% with maximal reduction at a magnesium concentration 10 times normal (11·3 mM).

Effect of potassium

Release of preformed protein from granules by exocytosis is usually stimulated by increasing the concentration of K^+ in the medium a phenomenon believed to be related to membrane depolarization. As shown in Table 3, increasing the concentration of K^+ in the medium led to an increase in the rate of release of amylase from guinea-pig pancreas. This increase at the highest concentrations of K^+ tested (94 mM) was similar to that observed in response to 10^{-4}M bethanechol. The responses to bethanechol and the increased concentration of K^+ in the medium, however, were not additive.

Argent, Case & Scratchard (1971) have recently reported that the K^+-stimulated release of amylase from perfused cat pancreas is blocked by atropine. They also presented evidence that K^+ acted by releasing endogenous acetylcholine. We have confirmed that 5×10^{-6}M atropine will block the release of amylase in response to a high concentration of K^+ in the medium, just as it is able to block the response to bethanechol, as shown in Table 4. Thus, if K^+ is acting by membrane depolarization it may well be the nerve terminal rather than the pancreatic acinar cell membrane which is the important site. This would also account for the lack of additive effect in the response to high K^+ and bethanechol.

TABLE 3. *Effect of increasing the concentration of K^+ in the medium on basal and bethanechol-stimulated amylase release from guinea-pig pancreas* in vitro

	Amylase release ((μg/mg)/h) Basal	10^{-4} M Bethanechol
KHB	0·75±0·03 (11)	1·18±0·07 (11)
×5 K^+	0·89±0·04 (11)*	1·14±0·07 (11)
KHB	0·78±0·02 (12)	1·33±0·05 (12)
×10 K^+	1·06±0·04 (12)**	1·18±0·01 (12)*
KHB	0·77±0·22 (14)	1·21±0·05 (14)
×20 K^+	1·22±0·04 (15)**	1·22±0·06 (15)

All values are the mean±S.E. of the number of determinations shown in parentheses on tissue from 5 animals. * $P<0·05$ as compared to response in KHB. ** $P<0·01$ as compared to response in KHB. The normal K^+ concentration is 4·7 mM.

TABLE 4. *Effect of atropine on (A) bethanechol- and (B) K^+-stimulated amylase release from guinea-pig pancreas* in vitro

	Amylase release ((μg/mg)/h)	
(A) Atropine (M)	Basal	10^{-4} M Bethanechol
0	0·62±0·01 (16)	0·94±0·03 (17)*
5×10^{-6}	0·73±0·04 (17)	0·64±0·03 (17)
(B) Atropine (M)	Basal	×10 K^+
0	1·05±0·05 (9)	1·41±0·09 (9)*
5×10^{-6}	1·05±0·11 (9)	1·07±0·09 (9)

All values are the mean±S.E. of the number of determinations shown in parentheses using tissue from 5 animals (bethanechol) and 3 animals (K^+). * $P<0·01$ as compared to basal release without atropine.

Cyclic AMP and amylase release

The possible role of cyclic AMP in mediating pancreatic amylase release was studied both by adding cyclic AMP to the medium and by measuring pancreatic cyclic AMP levels. As shown in Table 5 both cyclic AMP and dibutyryl cyclic AMP failed to stimulate amylase release in experiments in which a normal response to pancreozymin was obtained.

Pancreatic cyclic AMP concentrations were measured at times from 2 min to 60 min after exposure to bethanechol, pancreozymin and secretin. No increase over basal concentrations was seen. In the presence of 3 mM theophylline, however, secretin at a concentration of 0·5 U/ml increased cyclic AMP levels while pancreozymin did not (Fig. 3). Table 6 shows that 3 mM theophylline, which alone produced a slight rise ($P<0·05$) in pancreatic cyclic AMP, did not potentiate the response to bethanechol, pancreozymin or high K^+ in a manner similar to that of secretin. Neither theophylline alone nor theophylline and secretin together

TABLE 5. *Effect of cyclic AMP, dibutyryl cyclic AMP and pancreozymin on amylase release from guinea-pig pancreas* in vitro

Medium	Amylase release ((μg/mg)/h)	
KHB alone	1·09±0·07	(10)
3 mM cyclic AMP	1·19±0·09	(8)
10 mM cyclic AMP	1·22±0·12	(8)
0·1 mM dibutyryl cyclic AMP	0·98±0·08	(7)
1·0 mM dibutyryl cyclic AMP	1·25±0·12	(8)
300 mU/ml pancreozymin	2·08±0·39	(8)*

All values are the mean±S.E. of the number of determinations shown in parentheses with tissue from 4 or 5 animals. * $P<0·05$ as compared to response in KHB.

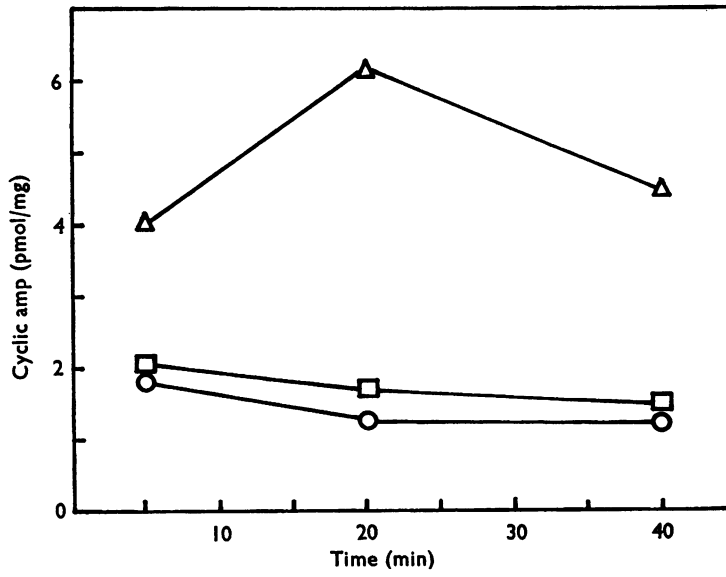

FIG. 3. Effects of secretin and pancreozymin on pancreatic cyclic AMP in the presence of theophylline. The cyclic AMP content of guinea-pig pancreas *in vitro* is plotted against time: (○——○), unstimulated; (□——□), 1,000 mU/ml pancreozymin plus 3 mM theophylline; (△——△), 500 mU/ml secretin plus 3 mM theophylline. Points are the means of duplicate determinations with tissue from a single pancreas.

stimulated amylase release (Table 7). Furthermore, theophylline did not potentiate the response to pancreozymin. Therefore, the rise in pancreatic cyclic AMP in response to theophylline and secretin combined is clearly not related to amylase release.

Effect of colchicine

The effect of colchicine on the basal and stimulated release of amylase is shown in Table 8. One hour preincubation in a medium containing 10^{-4}M colchicine was used because of the slow onset of secretory inhibition in other systems (Williams & Wolff, 1970; Malaisse, Malaisse-Lagae, Walker & Lacy, 1971). Even with this high concentration of colchicine, no inhibitory effect was seen on the basal, bethanechol- or pancreozymin-stimulated release of amylase.

Discussion

The present studies have examined factors involved in the control of pancreatic amylase secretion. Preformed zymogen granules containing amylase exist in pancreatic acinar cells and, over the short term (e.g. 60 min), control of their release or 'secretion' is separable from synthesis (Meldolesi, 1970; Morisset & Webster, 1971). Morphological studies have shown that zymogen granule contents are released by the process of exocytosis (Palade, Siekevitz & Caro, 1962).

TABLE 6. *The effects of pancreatic stimulators on pancreatic cyclic AMP concentrations in the presence and absence of theophylline*

	Cyclic AMP (pmol/mg)	
Medium	Theophylline 0	Theophylline 3 mM
KHB alone	0·9±0·06	1·2±0·06*
0·5 U/ml secretin	1·0±0·07	6·0±0·53**
1·0 U/ml pancreozymin	0·7±0·03	1·2±0·06*
10^{-4} M bethanechol	1·0±0·06	1·3±0·17
×10 K^+	0·9±0·07	1·3±0·10*

Pancreatic pieces were preincubated 15 min in KHB and then incubated in the specified medium for 10 min. All values are the mean±S.E. of 6 determinations with tissue from 3 animals. * $P<0·05$ as compared to KHB. ** $P<0·01$ as compared to KHB.

TABLE 7. *Effect of theophylline on amylase release in the presence of pancreozymin and secretin from guinea-pig pancreas* in vitro

	Amylase release (μg/mg)/h)		
Theophylline (mM)	KHB	Pancreozymin (500 mU/ml)	Secretin (500 mU/ml)
0	0·81±0·03	1·14±0·04*	0·90±0·05
3	0·73±0·03	1·11±0·04*	0·98±0·04

All values are the mean±S.E. of 8 determinations with tissue from 4 animals. * $P<0·01$ as compared to release in KHB alone.

TABLE 8. *Effect of colchicine on pancreozymin and bethanechol-stimulated amylase release from guinea-pig pancreas* in vitro

	Amylase release ((μg/mg)/h)		
Colchicine (M)	KHB	Pancreozymin (300 mU/ml)	Bethanechol (10^{-4} M)
0	0·72±0·06	1·00±0·05*	1·16±0·09*
10^{-4}	0·64±0·06	1·06±0·07*	1·23±0·10*

All values are the mean±S.E. of 8 determinations with tissue from 4 animals. * $P<0·01$ as compared to release in KHB.

The dependence of the exocytic process on ions such as Ca^{++} has been studied by Douglas and his colleagues and given the name of stimulus-secretion coupling, primarily as a result of work on the adrenal medulla and neurohypophysis (Douglas & Rubin, 1961; Douglas & Poisner, 1964; Douglas, 1968). In this scheme the stimulating agent induced an increased membrane permeability to Ca^{++} and Na^{+} with a resulting cation influx and membrane depolarization. Interest has focussed on the role of Ca^{++} in promoting exocytosis, possibly by an interaction with the granule/cell membrane (see Matthews, 1970). Secretion of a number of other hormones and enzymes stored as granules including those from the islets of Langerhans, adenohypophysis and salivary gland is consistent with this model (Geschwind, 1969; Rubin, 1970). By contrast, secretion of hormones from the adrenal cortex (Matthews & Saffran, 1972) and the thyroid gland (Williams, 1972), which occur by morphologically distinct processes, do not fit the model.

Evidence in support of a Ca^{++}-dependent stimulus-secretion coupling for the release of amylase from the pancreas includes the following: (1) Secretory stimuli, including cholinergic agents and pancreozymin, depolarize pancreatic cells (Dean & Matthews, 1972). (2) Removal of extracellular Ca^{++} blocks secretion. Hokin (1966) showed that amylase release from pigeon pancreas was blocked by presoaking in Ca^{++}-free medium containing EDTA. Recently, it has been shown that superfusion of pancreatic fragments by Ca^{++}-free medium diminishes secretion of lipase and amylase, as well as the response to carbamylcholine and pancreozymin (Robberecht & Christophe, 1971). In the present studies, removal of Ca^{++} completely blocked the response to bethanechol without affecting basal amylase release. (3) Elevation of the Mg^{++} concentration in the medium reduces the response to bethanechol. (4) Pancreozymin and cholinergic agents have recently been shown to increase the efflux of ^{45}Ca from pancreas *in vitro* (Case & Clausen, 1971). This observation may represent either release of intracellular Ca^{++} or an increase in membrane permeability to Ca^{++}.

Evidence which conflicts with the original model of stimulus-secretion coupling is that the K^{+} stimulation of amylase release is blocked by atropine as reported by Argent *et al.* (1971) and confirmed by us. Thus, in the exocrine pancreas the stimulation by K^{+} would appear to be mediated by release of endogenous transmitter. This is not the case for pancreozymin stimulation which is not blocked by atropine (Hokin & Hokin, 1962; Kulka & Sternlicht, 1968). Similar data have been presented for K^{+} stimulation of salivary amylase release (Schramm, 1968). These findings bring into question the role of membrane depolarization since atropine does not block the K^{+}-induced depolarization of pancreatic acinar cells (O. H. Petersen, personal communication). Of importance is the fact that cells of the adrenal medulla and neurohypophysis are of neural origin, whereas most other glandular cells, including those of the pancreas, are not. While neural cells are characterized by voltage dependent permeability changes, non-neural secretory cells may not possess this property. Thus the failure of acinar membrane depolarization by K^{+} *per se* to induce pancreatic secretion suggests that depolarization is an effect, rather than the cause, of increased cation permeability. While secretory depolarization is a readily measured signal of membrane events, its lack of causal importance may have to be considered.

Separate from the model of stimulus-secretion coupling involving Ca^{++}, but

perhaps related to exocytosis, has been the suggestion of a role for microtubules. Based primarily on the finding that the microtubular active agents, colchicine and the Vinca alkaloids, inhibit secretion by pancreatic islet cells (Lacy *et al.*, 1968, Malaisse *et al.*, 1971) and the adrenal medulla (Poisner & Bernstein, 1971), it has been speculated that microtubules might either guide granules to the membrane or function as a contractile link. However, colchicine does not block release of salivary amylase and hormones of the adenohypophysis (Kraicer & Milligan, 1971 ; Lockhart-Ewart & Taylor, 1971 ; Temple *et al.*, 1972). The present finding that colchicine does not affect pancreatic amylase release suggests that microtubules are not involved in this process. Thus microtubules do not appear to be necessarily invoked as a basic mechanism for stimulus-secretion coupling in all cells with regard to granular release. Alternative mechanisms exist (see Matthews, 1970).

While usually considered separately from the stimulus-secretion coupling model of Douglas, much effort has been devoted to establishing a role for cyclic AMP as a mediator of hormone and enzyme release. There is considerable overlap between systems in which Ca^{++} and cyclic AMP have been implicated, but the relative importance of these mechanisms remains to be established (Rasmussen, 1970). While Schramm and his co-workers (Badad, Ben-Zvi, Bdolah & Schramm, 1967) have presented evidence for a role of cyclic AMP in adrenaline-stimulated parotid amylase release, evidence for a pancreatic role is less complete. In the present study cyclic AMP and dibutyryl AMP did not lead to a statistically significant increase in amylase release. Cyclic AMP or its dibutyryl derivative have been reported by others to stimulate amylase release from pancreas *in vitro* (Kulka & Sternlicht, 1968; Ridderstap & Bonting, 1969 ; Morisset & Webster, 1971) but usually with much less effectiveness than pancreozymin. The phosphodiesterase inhibitor theophylline has similarly been reported to stimulate release (Kulka & Sternlicht, 1968 ; Ridderstap & Bonting, 1969), but only at concentrations which produce release by damage in other systems (Williams & Wolff, 1971). Furthermore, theophylline does not potentiate pancreozymin-stimulated release (present work ; Ridderstap & Bonting, 1969). Relatively minor effects of cyclic AMP and theophylline on chymotrypsin and lipase release from rat pancreas have also been reported recently (Bauduin *et al.*, 1971).

If cyclic AMP were of importance in mediating the stimulation of amylase release, substances which stimulate amylase release should increase pancreatic cyclic AMP levels. This was not found to be the case, whereas the combination of secretin plus theophylline, which was found to increase cyclic AMP levels, had no effect on amylase release. Since the pancreas does contain multiple cell types, the secretin stimulated rise in cyclic AMP may involve duct cells or cells other than acinar cells. It seems unlikely, however, that a significant effect on acinar cells which form the great bulk of the pancreas, would not have been apparent. While the present data do not rule out a possible contribution of cyclic AMP to pancreatic amylase release, it would not appear to be of major importance.

It is of interest that no data have appeared on a role for cyclic AMP in adrenal medullary secretion, the prototype tissue for the study of granule release. Furthermore, the role of cyclic AMP in promoting insulin release has recently been questioned (Montague & Cook, 1971). Perhaps the best data for a definite role of cyclic AMP in secretion are on thyroxine and corticosteroid hormone liberation (Schell-Frederick & Dumont, 1970 ; Garren, Gill, Masui & Walton, 1971), pro-

cesses which do *not* occur in a manner analogous to those in the exocrine pancrease and the adrenal medulla.

At present, therefore, pancreatic amylase release would appear to fit a version of the stimulus-secretion coupling model proposed by Douglas (1968) with major importance focussed on Ca^{++} flux.

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