The Metabolism of Cyclic Nucleotides in the Guinea-Pig Pancreas

ADENYLATE CYCLASE AND GUANYLATE CYCLASE

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Adenylate cyclase from the guinea-pig pancreas was activated in a dose-dependent manner by both secretin and cholecystokinin-pancreozymin, but in contrast with results in other species the hormones were approximately equipotent. All other hormones and transmitter substances tested were without any effect on adenylate cyclase activity. Guanylate cyclase activity was shown to have both particulate and supernatant components in the guinea-pig pancreas. The particulate enzyme, but not the supernatant enzyme, was markedly activated by Triton X-100, and most of the induced activity was released into the supernatant. The supernatant enzyme was specifically Mn^{2+} -dependent, but, even though Mn^{2+} was maximally effective at a concentration of 3 mM, activity could be raised further by increasing Ca^{2+} concentration. The particulate enzyme, by contrast, was relatively Mn^{2+} -independent. Activity of the particulate guanylate cyclase was enhanced by phosphatidylserine. The supernatant enzyme displayed classical Michaelis–Menten kinetics, but the particulate enzyme deviated markedly from such kinetics. Under none of the conditions used was any significant activation of guanylate cyclase observed with any of the secretogen hormones or transmitter substances.

There is substantial evidence that the rise in tissue cyclic AMP concentration resulting from the action of hormones is due to activation of adenylate cyclase in the membrane of the target cell. In the pancreas there is good correlation between the action of secretin in raising cyclic AMP concentration *in vitro* (Albano *et al.*, 1976; Benz *et al.*, 1972; Lopatin & Gardner, 1978) and *in vivo* (Case *et al.*, 1972), and activation of adenylate cyclase in broken-cell preparations (Rutten *et al.*, 1972; Lemon & Bhoola, 1975). There is a less-clear relationship with cholecystokinin-pancreozymin: pancreatic cyclic AMP concentrations are raised by cholecystokinin-pancreozymin in the cat (Case *et al.*, 1972) and in the rat (Kempen *et al.*, 1977) but not in the guinea-pig (Albano *et al.*, 1976; Benz *et al.*, 1972; Lopatin & Gardner, 1978), and furthermore the sensitivity of adenylate cyclase to this hormone in pancreatic homogenates also varies with species (Rutten *et al.*, 1972; Lemon & Bhoola, 1975).

No activation of guanylate cyclase, analogous to the activation of adenylate cyclase by hormones, has yet been demonstrated to account for the rapid rise in pancreatic cyclic GMP concentration caused by

acetylcholine, cholecystokinin-pancreozymin and substance P (Albano *et al.*, 1976; Haymovitz & Scheele, 1976; Lopatin & Gardner, 1978; Albano *et al.*, 1978).

In the present paper we delineate the biochemical properties and report on the action of hormones on adenylate cyclase and guanylate cyclase, and consider the possible physiological mechanisms by which the activity of guanylate cyclase in the guinea-pig pancreas might be modulated by these hormones.

Materials

All radioisotope-labelled materials were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Triton X-100, ATP (trisodium salt) and acetylcholine hydrochloride were obtained from Sigma. Carbachol was purchased from BDH. Cholecystokinin-pancreozymin and secretin were obtained from GIH Laboratories, Stockholm, Sweden. Substance P was from CIBA–Geigy. Phosphatidylserine was extracted from fresh rat brain as described by Saunders (1967).

Methods

Preparation of guanylate cyclase

Guinea-pigs (non-starved adult males) were killed by cervical dislocation. The pancreas was removed, freed of fat and blood vessels, and minced with fine scissors. The tissue from three animals was homogenized in a glass hand homogenizer in 5 vol. of 10 mM-Tris/HCl buffer, pH 7.5, containing 0.1 mM-EDTA. The homogenate was filtered through a single layer of nylon mesh, and the filtrate centrifuged for 1 h at 100 000 g. The supernatant was decanted and used directly as the source of enzyme. The enzyme was kept at 0–4°C throughout its preparation, and always freshly prepared for assay.

Preparation of adenylate cyclase

Filtered homogenate prepared as for guanylate cyclase was centrifuged for 20 min at 1500 g, and the pellet resuspended in 5 vol. of homogenizing buffer. This was centrifuged again for 20 min at 1500 g, and the pellet suspended in homogenizing buffer to give a concentration of 10–20 mg of protein/ml.

Preparation of triethylammonium bicarbonate

Triethylamine (200 ml) was mixed with 400 ml of distilled water, and excess CO₂ (released from a flask containing solid CO₂) allowed to bubble through the mixture until the two phases became a clear homogeneous solution. This was diluted to give the appropriate concentration of triethylammonium bicarbonate (calculated from the original amount of triethylamine).

Purification of [α -³²P]ATP and [α -³²P]GTP by anion-exchange chromatography

This was achieved chromatographically by a modification of the method of Cha & Parks (1964). Commercially supplied [α -³²P]ATP or [α -³²P]GTP (1 mCi; specific radioactivity about 10 Ci/mmol) was dissolved in 1 ml of 0.3 M-triethylammonium bicarbonate and loaded on to a column (1.2 cm × 10 cm) of DEAE-Sephadex A-25 equilibrated with 0.3 M-triethylammonium bicarbonate (0.3–1.0 M, 120 ml). Fractions (1.8 ml) were collected at a flow rate of 40 ml/h. The fractions containing the major peak of radioactivity (eluted after about 90 ml) were pooled, freeze-dried and the residues dissolved in 2 mM-ATP or -GTP for storage at –20°C. Assay blanks did not deteriorate in samples drawn from this stock over a period of 3 weeks.

Assay of adenylate cyclase and guanylate cyclase with the use of alumina columns

The incubation mixture (total volume 0.1 ml) for guanylate cyclase consisted of 1 mM-cyclic GMP, 3 mM-MnCl₂, 3 mM-CaCl₂, 20 mM-phosphoenolpyruvate, 60 µg of pyruvate kinase/ml (Boehringer,

Mannheim, West Germany), 2 mM-glutathione and 0.22 mM-[α -³²P]GTP (about 6 × 10⁵ d.p.m. total radioactivity) in 40 mM-Tris/HCl, pH 7.5. The reaction was initiated by addition of 20 µl of the enzyme preparation. For adenylate cyclase, the incubation mixture (total volume 80 µl) consisted of 2 mM-cyclic AMP, 5.5 mM-KCl, 15 mM-MgSO₄, 20 mM-phosphoenolpyruvate, 60 µg of pyruvate kinase/ml and 0.5 mM-[α -³²P]ATP (about 6 × 10⁵ d.p.m. total radioactivity) in 40 mM-Tris/HCl, pH 7.5. The reaction was initiated by addition of 10 µl of the enzyme preparation. For both guanylate cyclase and adenylate cyclase the incubation was carried out in a shaking water bath for 15 min at 37°C. The incubation was terminated by addition of 20 µl of 30% (w/v) trichloroacetic acid, and 10 µl of the appropriate ³H-labelled cyclic nucleotide (about 6 × 10⁴ d.p.m.) was added as an internal recovery standard. Each sample was neutralized with 20 µl of 1.5 M-NaOH and 0.2 ml of 0.4 M-Tris/HCl, pH 7.0, and centrifuged for 5 min at 5000 g to remove any precipitated material. The supernatant was pipetted off and applied to an alumina column (chromatographic alumina; 1.5 g dry wt.; May and Baker) to achieve separation of cyclic nucleotides from other nucleotides (White & Zenser, 1971) with 0.4 M-Tris/HCl as the eluting agent as previously described (Methven *et al.*, 1979). It was found that 0.2 M-Tris/HCl buffer gave slightly lower blanks with [α -³²P]ATP than did 0.4 M-Tris/HCl, and hence the lower concentration was used to equilibrate and elute the alumina columns in the adenylate cyclase assay.

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard.

Presentation of data

Each experimental value represents an assay performed on three portions taken from an enzyme sample prepared from the pooled pancreatic tissue of three guinea pigs. The triplicate measurements, which were always in good agreement, were subjected to statistical analysis, and error values determined.

Definition of biological units of secretin and cholecystokinin-pancreozymin

One clinical unit of secretin when administered intravenously in healthy individuals as a dose of 1 clinical unit/kg body wt. produces pancreatic juice of volume, bicarbonate concentration and amylase secretion within a defined range of values. One Ivy dog unit is defined as the amount of freeze-dried hormone that, when dissolved in 0.9% NaCl and injected intravenously during a 10–15 s period into an anaesthetized dog weighing about 15 kg, results within 3 min in a rise in intra-gall-bladder pressure of

1 cm of bile (Jorpes & Mutt, 1973). The biological potencies of secretin and cholecystokinin-pancreozymin are sufficiently similar to be compared in the nucleotide cyclase assay. One unit of each hormone is standardized by bioassay to give specific and definable physiological responses, so that 1 mg of pure secretin (mol.wt. 3055) has the biological activity of 4000 clinical units, and 1 mg of pure cholecystokinin-pancreozymin (mol.wt. 3919) has the biological activity of 3000 Ivy dog units.

Results

Adenylate cyclase

Subcellular distribution. The particulate nature of pancreatic adenylate cyclase was confirmed in subcellular-distribution experiments (Table 1). The 1500g pellet contained most of the activity recovered in the fractions, and the specific activity was also the highest. Hence the 1500g pellet was used as the source of adenylate cyclase in subsequent experiments.

Temperature-stability of adenylate cyclase. The stability of adenylate cyclase was investigated by incubating freshly prepared enzyme at 20°C, 4°C and 0°C for 45 min before assay. The effect of including phosphatidylserine (1 mM) during this preincubation period was also determined, since phospholipids have been shown to play an important role in cholecystokinin-pancreozymin-induced activation of pancreatic adenylate cyclase (Kempen *et al.*, 1977; Svobada *et al.*, 1976). The results (Table 2) show that basal and secretin- and cholecystokinin-pancreozymin-stimulated activities remained highest when the enzyme was kept at 4°C. The inactivation process which resulted in rapid loss of activity at 20°C appears to be prevented by some component of the assay medium, since activity remains constant over a 30 min time course at 37°C.

Activation by secretin and cholecystokinin-pancreozymin. The hormone secretagogues, secretin and cholecystokinin-pancreozymin, both stimulated adenylate cyclase in a dose-dependent manner (Fig. 1). Phosphatidylserine (0.13 mM) was slightly inhibi-

Table 1. *Subcellular distribution of adenylate cyclase in the guinea-pig pancreas*

The specific activities of fractions produced by differential centrifugation of a homogenate of guinea-pig pancreas are tabulated as means $\pm$ S.E.M. for triplicate determinations. The total activities expressed as percentages of the homogenate activities are recorded in parentheses. The recovery of lactate dehydrogenase (assayed as a cytochemical marker as described by Bergmeyer *et al.*, 1963) is also recorded. ND, Not detectable.

	Adenylate cyclase activity (pmol of cyclic AMP/min per mg of protein)			Lactate dehydrogenase activity
	Basal	+Secretin (5 clinical units/ml)	+Fluoride (7.5 mM)	
Homogenate	6.8 $\pm$ 0.6 (100)	12.5 $\pm$ 3.7 (100)	101.1 $\pm$ 10.5 (100)	(100)
20 min $\times$ 1500 g pellet	17.8 $\pm$ 7.4 (64.1)	39.8 $\pm$ 5.8 (78.8)	155.1 $\pm$ 10.3 (37.5)	(6.3)
60 min $\times$ 100 000 g pellet	11.6 $\pm$ 0.2 (21.1)	15.1 $\pm$ 0.4 (10.5)	139.2 $\pm$ 2.3 (12.0)	(6.0)
60 min $\times$ 100 000 g supernatant	ND (0)	ND (0)	7.8 $\pm$ 2.0 (3.2)	(89.0)

Table 2. *Effect of preincubation on adenylate cyclase activity*

Activities of adenylate cyclase were measured after preincubation of the enzyme for 45 min in the presence and absence of 1 mM-phosphatidylserine as described in the text. Results are given as means $\pm$ S.E.M. for triplicate determinations. ND, Not detectable.

Temperature of preincubation (°C)	Presence of phosphatidylserine	Adenylate cyclase activity (pmol of cyclic AMP/min per mg of protein)			
		Basal	+Secretin (2 clinical units/ml)	+Cholecystokinin -pancreozymin (2 Ivy dog units/ml)	+Fluoride (7.5 mM)
0	—	11.8 $\pm$ 0.5	25.6 $\pm$ 0.9	31.2 $\pm$ 1.5	87.3 $\pm$ 7.2
0	+	8.9 $\pm$ 1.0	21.8 $\pm$ 0.3	24.4 $\pm$ 2.9	Not assayed
4	—	14.0 $\pm$ 2.2	29.8 $\pm$ 2.3	38.9 $\pm$ 3.8	79.2 $\pm$ 1.9
4	+	11.3 $\pm$ 0.3	25.0 $\pm$ 1.1	20.4 $\pm$ 2.0	Not assayed
20	—	ND	ND	ND	8.2 $\pm$ 0.4
20	+	ND	ND	ND	Not assayed

Fig. 1. Activation of adenylate cyclase by secretin and cholecystokinin-pancreozymin

Adenylate cyclase activity in the 1500g-pellet fraction from guinea-pig pancreas was measured in the presence of different concentrations of secretin (○) and cholecystokinin-pancreozymin (●), expressed in clinical units/ml and Ivy dog units/ml respectively. Results are expressed as means of triplicate determinations, and the vertical bar associated with each symbol represents 1 S.E.M.

tory when included in the incubation mixture: basal and secretin (2 clinical units/ml)-stimulated activities were not significantly affected, but cholecystokinin-pancreozymin (5 Ivy dog units/ml)- and fluoride (7.5 mM)-stimulated activities were reduced by 20 and 23% respectively. Noradrenaline, carbachol, acetylcholine, 5-hydroxytryptamine and histamine (all tested at 0.1 mM) and substance P (tested at 12.5 μM) were without any effect on the activity of adenylate cyclase from the guinea-pig pancreas.

Guanylate cyclase

Subcellular distribution (Fig. 2). In contrast with adenylate cyclase, most of the guanylate cyclase activity (74% of total recovered activity) was found in the 100000g supernatant fraction. If the tissue was homogenized and centrifuged in the presence of 1% Triton X-100, there was a 2-fold increase in guanylate cyclase activity in the homogenate and a 2.5-fold increase in activity in the 100000g super-

natant. This appears to represent a release into solution of enzyme latent as an inactive membrane-bound form, since 1% Triton X-100 included in the incubation medium did not stimulate the supernatant enzyme. It was important to determine whether the activity associated with the pellet fractions represents a true membrane-associated enzyme or merely soluble enzyme trapped in the pellet during centrifugation. Although such trapping must certainly account for some of the pellet activity, it was found that, whereas only 6.3% of a cytoplasmic marker (lactate dehydrogenase) was present in the 1500g pellet, it contained at least 18% of the total guanylate cyclase activity. If the pancreatic tissue was homogenized by sonic disruption (2×15 s, at 8 mHz in an MSE ultrasonic disintegrator with a titanium probe) instead of mechanically, the subcellular distribution was unaltered.

Effect of Triton X-100. Non-ionic detergents increase the activity of particulate guanylate cyclase (Ishikawa *et al.*, 1969; White & Aurbach, 1969). Soluble guanylate cyclase in some tissues is also activated by non-ionic detergents, although invariably to a much smaller extent. In the guinea-pig pancreas, the 100000g supernatant guanylate cyclase was unaffected by 1% Triton X-100, whereas the 1500g-pellet enzyme was activated in a dose-dependent manner (Fig. 3).

Effects of Mg^{2+} , Mn^{2+} and Ca^{2+} . When Mn^{2+} and Ca^{2+} were omitted from the incubation medium, the activity of supernatant guanylate cyclase was very low (0.5 ± 0.1 pmol/min per mg of protein) and was undetectable if 0.1 mM-EDTA was included in the incubation. Mn^{2+} (3 mM) in the incubation medium caused a marked increase in activity, but this could be increased still further by raising the Ca^{2+} concentration to 3 mM. The enzyme was almost entirely Mn^{2+} -specific, and replacement of Mn^{2+} by Mg^{2+} was ineffective (Fig. 4). Heparin (500 i.u./ml) almost completely inhibited supernatant guanylate cyclase. This is probably due to the powerful chelator action of heparin. Interestingly, particulate guanylate cyclase did not possess the same Mn^{2+} -dependence as the supernatant enzyme. At GTP concentrations of both 0.2 and 0.1 mM, the activity of the 1500g-pellet fraction was not altered by raising the Mn^{2+} concentration from 0.1 to 3 mM.

Effect of ATP. ATP is a potent inhibitor of supernatant guanylate cyclase. At a GTP concentration of 0.22 mM, 0.1 mM-ATP had no effect on guanylate cyclase, but inhibition by 0.5 mM- and 2.0 mM-ATP was 32.8 ± 2.0 and $93.9 \pm 2.0\%$ respectively.

Kinetic characteristics. Supernatant guanylate cyclase demonstrated almost classical Michaelis-Menten kinetics (Fig. 5). The reaction rates were constant over 30 min at GTP concentrations of 200 μM. Even at the lowest concentrations of GTP used and under conditions of maximum activity (i.e.

Fig. 2. Subcellular distribution of guanylate cyclase activity

Guinea-pig pancreatic homogenate (H) was centrifuged to yield a $20 \text{ min} \times 1500g$ pellet (P1), a $60 \text{ min} \times 10000g$ pellet (P2) and a $60 \text{ min} \times 100000g$ supernatant (S). Triton X-100 was included as follows: ■, Control; ▨, 1% Triton X-100 in the assay medium; ▩, 1% Triton X-100 in the homogenizing buffer; ▤, 1% Triton X-100 in both homogenizing buffer and incubation medium. Histograms are the means of triplicate determinations of total activity in each fraction, with the s.e.m. represented by the vertical bar.

Fig. 3. Effect of Triton X-100 on particulate guanylate cyclase activity

The activity of guanylate cyclase (●) in the $20 \text{ min} \times 1500g$ pellet was measured in the presence of Triton X-100 up to a concentration of 5% (w/v).

Fig. 4. Effects of Mn^{2+} , Mg^{2+} and Ca^{2+} on the activity of supernatant guanylate cyclase

The activity of guanylate cyclase in supernatant fraction from guinea-pig pancreas was measured in incubation media in which the concentrations of Mn^{2+} , Mg^{2+} and Ca^{2+} were varied. The effects of increasing the Mn^{2+} concentration in the presence of 3 mM-Ca^{2+} (■), of increasing the Mg^{2+} concentration in the presence of 3 mM-Ca^{2+} (○), and of increasing the Ca^{2+} concentration in the presence of 3 mM-Mn^{2+} (●) or 0.1 mM-Mn^{2+} (□) are plotted as means $\pm$ s.e.m. for triplicate determinations.

Results are given as means $\pm$ s.e.m. for triplicate determinations.

Fig. 5. Plot of reciprocal of GTP concentration for guanylate cyclase

The 1500g pellet (●, no Triton X-100; ○, in the presence of 1% Triton X-100) and 100000g supernatant (■, no Triton X-100; □, in the presence of 1% Triton X-100) guanylate cyclase activities were measured at GTP concentrations in the range 0.02–1.02 mM. The results, which are means of triplicate determinations, are plotted as reciprocals of activity against reciprocal of GTP concentration.

with the supernatant enzyme), conversion of GTP to cyclic GMP reaches less than 0.1% during the 15 min incubation period. The GTP-regenerating system was effective in maintaining GTP concentrations, under the assay conditions used, and therefore substrate depletion was not a limiting factor in our kinetic studies. Analysis by a linear-regression method (Wilkinson, 1961) of data obtained over the GTP concentration range 0.02–1.02 mM gave an apparent K_m of $94 \pm 26 \mu\text{M}$ in the absence of Triton X-100, and $61 \pm 18 \mu\text{M}$ in the presence of 1% Triton X-100. Guanylate cyclase in the 1500g pellet, on the other hand, did not follow Michaelis-Menten kinetics. A plot of the reciprocal of GTP concentration against the reciprocal of activity (Fig. 5) was non-linear, so that this enzyme cannot be considered in terms of K_m . However, 1% Triton X-100 caused a marked change in the double-reciprocal plot, shifting the values towards those obtained with the supernatant enzyme.

Effect of physiologically active substances on the particle-associated enzyme. The effects of hormones and transmitter substances was tested on the 1500g-pellet enzyme and not on the supernatant enzyme, since the substances are thought to act at the plasma membrane, and would not therefore be expected to have a direct effect on intracellular cytoplasmic enzymes. Secretin (2 clinical units/ml), cholecystokinin-pancreozymin (5 Ivy dog units/ml), acetylcholine (1 nM–10 μM), carbachol (1 nM–10 μM), nor-adrenaline (0.1 mM), histamine (0.1 mM), 5-hydroxy-

tryptamine (0.1 mM) and substance P (10 μM) were without significant effect on the enzyme. Fluoride up to a concentration of 7.5 mM was also without effect. When phosphatidylserine (0.2 mM) was included in the incubation medium the basal activity of guanylate cyclase was increased by 76%. In the presence of the various hormones and transmitter substances, the increase in the activity when phosphatidylserine was introduced into the incubation medium ranged from 16 to 83%, but in no case was it significantly greater than the control activity. The possibility was considered that activation of guanylate cyclase consists of increasing the affinity of the enzyme for GTP. Since the K_m of supernatant guanylate cyclase is less than 100 μM , it was considered possible that activation might be much more clearly seen at low GTP concentrations. However, no activation was observed with carbachol (0.1 and 10 μM), even when the GTP concentration was reduced to 0.02 mM.

Discussion

We have confirmed the exclusively particulate nature of adenylate cyclase and its dose-dependent activation by secretin in the guinea-pig pancreas. In the cat (Lemon & Bhoola, 1975) and in the rat (Rutten *et al.*, 1972), adenylate cyclase was much less sensitive to cholecystokinin-pancreozymin than to secretin, but this was not found to be so in the guinea pig. It is difficult to reconcile the inability of cholecystokinin-pancreozymin to alter cyclic AMP concentrations in guinea-pig pancreatic slices with the marked activation of adenylate cyclase by similar concentrations of the hormone in broken-cell preparations. We originally assumed this to be due to the inability of cholecystokinin-pancreozymin to reach the appropriate adenylate cyclase receptor in intact tissue, but the observation that cyclic AMP concentrations are unaffected even in isolated cell preparations (Lopatin & Gardner, 1978) refutes this interpretation. An alternative explanation may be a simultaneous stimulation of both adenylate cyclase and cyclic AMP phosphodiesterase, so that no net rise in tissue cyclic AMP concentration is detected.

Physiological studies (see Thomas, 1967) suggest that it is the centroacinar and duct cells that respond to secretin, and the acinar cells that respond to cholecystokinin-pancreozymin, but whether secretin- or cholecystokinin-pancreozymin-activated adenylate cyclases are located exclusively in a particular cell type cannot be resolved until homogeneous populations of the two cell types have been tested. In the rat (Kempen *et al.*, 1977) secretin-sensitive adenylate cyclase activity has been demonstrated on membrane fractions prepared from both cell types.

Soluble guanylate cyclase from the guinea-pig pancreas was markedly and specifically Mn^{2+} -

dependent, but the physiological importance of this highly specific activation by Mn^{2+} is difficult to assess, since the mean tissue concentration of Mn^{2+} in the pancreas is about $40\mu M$ (Fore & Morton, 1952). Similarly, the concentration of Ca^{2+} that enhanced the Mn^{2+} activation of the supernatant enzyme was far in excess of the $0.1\text{--}10\mu M$ Ca^{2+} considered to occur in the cytoplasm. Unless there is compartmentation it is difficult to envisage how Ca^{2+} can modulate cytoplasmic guanylate cyclase activity in intact cells. It is possible that the soluble guanylate cyclase normally exists in an inhibited state, and its conversion to a partially activated form may depend on complex metal interactions between Mn^{2+} , Ca^{2+} and Mg^{2+} , particularly since the intracellular concentration of Mg^{2+} is about 10^3 times higher than either those of the other two ions. Inhibition of the supernatant guanylate cyclase by ATP is probably important in the functional regulation of the enzyme (Kimura & Murad, 1975).

A particulate guanylate cyclase was identified in homogenates of the pancreas. In marked contrast with the supernatant enzyme the particulate guanylate cyclase showed no Mn^{2+} -dependence. It is possible that the particulate enzyme could be translocated into the soluble form, and, associated with such a change, an alteration occurs in its requirement for the different cations. The optimum ion-dependence appears also to differ with the source of enzyme. Membrane-bound guanylate cyclase prepared from rabbit skeletal muscle is in fact activated by Ca^{2+} but only at submaximal concentrations of Mn^{2+} (Chrisman *et al.*, 1975; Levine *et al.*, 1979).

Secretogens, which are known to raise cyclic GMP concentrations in intact pancreatic tissue, consistently failed to stimulate the particulate enzyme under our standard assay conditions. Alteration in GTP and Mn^{2+} concentrations in the assay medium failed to evoke stimulation of guanylate cyclase by carbachol. The inability of transmitters and hormones to activate membrane-bound guanylate cyclase may be due to a dissociation of essential cofactors in the cell-free system.

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