The Metabolism of Cyclic Nucleotides in the Guinea-Pig Pancreas

CYCLIC AMP PHOSPHODIESTERASE AND CYCLIC GMP PHOSPHODIESTERASE

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(Received 11 May 1979)

Both cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase were recovered mainly from the supernatant fractions of guinea-pig pancreas, but a higher proportion of the activity of the former was associated with the pellet fractions. The activities in the supernatant were not separated by gel filtration, but were clearly separated by subsequent chromatography on an anion-exchange resin. The activities of cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase had high-affinity (K_m $6.5 \pm 1.1 \mu M$ and $31.9 \pm 3.9 \mu M$ respectively) and low-affinity (K_m 0.56 ± 0.05 mM and 0.32 ± 0.03 mM respectively) components. The activity of neither enzyme was affected by the pancreatic secretogens, cholecystokinin-pancreozymin, secretin and carbachol. Removal of ions by gel filtration resulted in a marked reduction in cyclic nucleotide phosphodiesterase activity, which could be restored by addition of Mg^{2+} . Mn^{2+} (3 mM) was as effective as Mg^{2+} (3 mM) in the case of cyclic AMP phosphodiesterase, but was less than half as effective in the case of cyclic GMP phosphodiesterase. The metal-ion chelators, EDTA and EGTA, also decreased activity. Ca^{2+} (1 mM) did not affect the activity of cyclic nucleotide phosphodiesterase when the concentration of Mg^{2+} was 3 mM. At concentrations of Mg^{2+} between 0.1 and 1 mM, 1 mM- Ca^{2+} was activating, and at concentrations of Mg^{2+} below 0.1 mM, 1 mM- Ca^{2+} was inhibitory. These results are discussed in terms of the possible significance of cyclic nucleotide phosphodiesterase in the physiological control of cyclic nucleotide concentrations during stimulus-secretion coupling.

Both cyclic AMP (Kulka & Sternlicht, 1968; Morisset & Webster, 1971; Case & Scratcherd, 1972; Benz *et al.*, 1972) and cyclic GMP (Albano *et al.*, 1976; Haymovits & Scheele, 1976) have been implicated as chemical mediators in the secretory response of the exocrine pancreas to physiological stimuli. In the case of cyclic AMP, it has been generally accepted that the primary membrane event is activation of adenylate cyclase by secretin (Rutten *et al.*, 1972; Lemon & Bhoola, 1975). However, a clear and reproducible hormone-induced activation of guanylate cyclase analogous to activation of adenylate cyclase has yet to be convincingly demonstrated, despite the fact that pancreatic cyclic GMP concentrations are markedly and specifically raised by certain hormone secretagogues (Albano *et al.*, 1976, 1978; Haymovits & Scheele, 1976; Lopatin & Gardner, 1978). In the present paper we report a detailed study of the properties of cyclic nucleotide phosphodiesterases and consider the possibility that modulation of these enzymes may be an important factor in physiological

regulation of cyclic nucleotide concentrations in the pancreas.

Materials

All radioisotope-labelled materials were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Cyclic AMP and cyclic GMP were obtained as the sodium salts from Sigma. Secretin and cholecystokinin-pancreozymin were obtained from GIH Laboratories, Stockholm, Sweden, and bovine pancreatic trypsin inhibitor (Trasylol) was kindly supplied by Bayer. The phosphodiesterase inhibitors (papaverine, isobutylmethylxanthine, theophylline and caffeine) were obtained from Sigma.

Methods

Preparation of cyclic nucleotide phosphodiesterase

Guinea pigs (non-starved adult males, 300–400 g) were killed by cervical dislocation. The pancreas was removed, freed of fat and blood vessels, and minced with fine scissors. The tissue was homo-

genized in 8 vol. of 10 mM-Tris/HCl buffer, pH 7.4, containing 0.25 M-sucrose, in an MSE polytetrafluoromethylene-blade homogenizer at full speed (3×15 s, with 30 s intervals). The homogenate was filtered through a single layer of nylon mesh and the filtrate centrifuged at 300 000 g for 1 h. The decanted supernatant or the filtered homogenate was used as the source of enzyme. The entire preparation was carried out at 0–4°C, and only freshly prepared enzyme was used. Enzyme activity was measured in the 300 000 g supernatant fraction, and only where stated was activity in the 300 000 g pellet determined. In experiments in which the effect of addition of ions was studied, endogenous ions were first removed from the supernatant enzyme preparation by gel filtration: 3.5 ml of supernatant was loaded on to a column (12 cm $\times$ 3 cm) of Sephadex G-25 (Pharmacia) equilibrated with 50 mM-Tris/HCl buffer, pH 7.5, and the enzyme eluted with this buffer at a flow rate of 30 ml/h. The eluate was continuously monitored at 280 nm with a Uvicord spectrophotometer (LKB Products), and the protein-containing eluate (about 4 ml) was used as the source of ion-free enzyme. All procedures were performed at 0–4°C.

Assay of cyclic nucleotide phosphodiesterase with the use of alumina columns

The standard assay mixture (total volume 0.2 ml) contained 3 mM-MgCl₂, 1 mM-AMP or -GMP, 5 μ M-³H-labelled cyclic nucleotide (about 2×10^5 d.p.m./nmol) in 0.05 M-Tris/HCl buffer, pH 7.4. The reaction was started by the addition of 50 μ l of enzyme preparation. The incubation was carried out in a shaking water bath (Grant Instruments) at 37°C for 5–30 min, and terminated by heating the tubes for 3 min in an electrically heated aluminium block (Grant Instruments) set at 110°C. ¹⁴C-labelled cyclic nucleotide (25 μ l; 5×10^3 d.p.m.) was added to each tube immediately before boiling to act as an internal recovery standard. Cyclic nucleotide in the boiled sample was separated from other labelled products by the method of White & Zenser (1971). The samples were loaded on to individual alumina columns (chromatographic alumina; 1.5 g dry wt.; May and Baker) equilibrated with 0.4 M-Tris/HCl buffer, pH 7.0. Each tube was washed with 0.2 ml of the same buffer and the washings were added to the corresponding column. The columns were allowed to drain directly into scintillation vials and were eluted with 1.8 ml of column buffer. The eluted material was taken up in 18 ml of scintillation fluid [1.25 litres of Triton X-100, 15 g of 2,5-diphenyloxazole and 1 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene in 3.75 litres of xylene] and counted for radioactivity on a Packard Tri-Carb liquid-scintillation counter. ³H and ¹⁴C were calculated by a dual-channel method.

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 were determined.

Validation of the assay method

The assay was checked by chromatographing a 50 μ l sample of the boiled incubation mixture to which 10 μ l of a marker solution containing unlabelled cyclic nucleotide (5 mM), nucleoside 5'-monophosphate (5 mM) and nucleoside (5 mM) was added. A portion (10 μ l) of each sample was spotted on a poly(ethyleneimine)-cellulose-coated glass plate [20 cm $\times$ 20 cm; prepared as described by Randerath & Randerath (1967)] and run in 0.05 M-LiCl (in the case of cyclic GMP phosphodiesterase) or ethanol/0.5 M-ammonium acetate (2:1, v/v) (in the case of cyclic AMP phosphodiesterase). The plates were dried at room temperature, and viewed under u.v. light (253 nm). The regions containing marker material were scraped off and placed in individual scintillation vials. The nucleotides were eluted with 1 ml of 0.4 M-Tris/HCl buffer for 1 h, and the mixture was taken up in 9 ml of scintillation fluid and counted for radioactivity. In time-course and protein-dependence experiments, in which samples were processed by both the alumina and t.l.c. methods, results were identical. Furthermore, data from the t.l.c. method showed that both cyclic AMP and cyclic GMP were quantitatively converted to the appropriate nucleoside 5'-monophosphate, and that no other tritiated products were formed during the incubation.

Partial purification of cyclic nucleotide phosphodiesterase

Freshly prepared supernatant cyclic nucleotide phosphodiesterase (5 ml) was applied to a column (100 cm $\times$ 1.5 cm) of Ultragel AcA 22 (LKB Products) and eluted with 50 mM-Tris/HCl buffer, pH 7.5, containing 3 mM-MgCl₂ and 0.1 mM-EDTA. Fractions (9 ml) were collected at a flow rate of 30 ml/h. Fractions 9 to 16 inclusive (containing 74 and 76% respectively of the applied cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase) were combined and loaded on to a column (15 cm $\pm$ 5.5 cm) of DEAE-Sephadex A-50 (Pharmacia) equilibrated with the gel-filtration buffer. The column was washed with 150 ml of the buffer, and cyclic nucleotide phosphodiesterase activity was eluted on a KCl gradient (0–0.85 M) introduced

into the buffer. Fractions (18 ml) were collected at a flow rate of 12 ml/h. All procedures were carried out at 4°C.

Results

Subcellular distribution

The subcellular distribution of pancreatic cyclic nucleotide phosphodiesterase was examined in the absence and in the presence of 0.5% Triton X-100 in the homogenizing buffer (Table 1). The results show that the rate of hydrolysis of cyclic AMP was greater than that of cyclic GMP in all of the fractions. About 13% of the cyclic GMP-hydrolysing activity recovered in the fractions was associated with the pellet fractions, compared with 22% of the cyclic AMP-hydrolysing activity; 7.5% of the cytoplasmic marker enzyme, lactate dehydrogenase (assayed as described by Bergmeyer *et al.*, 1963), was found in the pellet fractions (3.3% in P1, 2.1% in P2 and 2.1% in P3; see Table 1). Inclusion of Triton X-100 (0.5%, w/v) in the homogenizing buffer resulted in a decrease in the proportion of the cyclic AMP phosphodiesterase activity recovered from the pellet fractions, but did not affect the distribution of cyclic GMP phosphodiesterase activity between pellet and supernatant fractions. Triton X-100 reduced the activity of cyclic AMP phosphodiesterase in the homogenate by 33%, but did not affect the cyclic GMP phosphodiesterase activity.

Effects of ions

It was considered important to characterize fully the effect of bivalent cations, particularly Ca^{2+} , on the supernatant cyclic nucleotide phosphodiesterases, since such information might reveal a possible mechanism for control of the intracellular enzyme by drugs and hormones. We first investigated the action of bivalent-cation chelators on activity, using

increasing concentrations of EDTA and EGTA in the presence of the standard 3 mM- Mg^{2+} (Fig. 1). Inhibition of cyclic AMP phosphodiesterase was not significant at concentrations below 1 mM, but at a concentration of 10 mM both chelators were markedly inhibitory. At concentrations of chelator below 1 mM, cyclic GMP phosphodiesterase was more strongly inhibited by EGTA than by EDTA, but, whereas 10 mM-EDTA almost completely inhibited the enzyme, activity was decreased by only 40% in the presence of 10 mM-EGTA.

The bivalent-cation requirement of cyclic nucleotide phosphodiesterases was further studied by titrating Mg^{2+} and Ca^{2+} against an ion-free enzyme preparation (Figs. 2 and 3). Cyclic AMP phosphodiesterase activity appeared to be independent of added Ca^{2+} above 1 mM- Mg^{2+} ; at low concentrations of Mg^{2+} , 1 mM- Ca^{2+} was inhibitory, but at intermediate concentrations, 1 mM- Ca^{2+} appeared to potentiate the effect of Mg^{2+} strongly. Cyclic GMP phosphodiesterase behaved similarly, except that Ca^{2+} was more inhibitory and Mn^{2+} was not completely effective as a substitute for Mg^{2+} .

Effects of cyclic nucleotides

Another possible intracellular modulator of cyclic nucleotide phosphodiesterase activity is the alternative cyclic nucleotide. Low concentrations of cyclic GMP have been shown to activate cyclic AMP phosphodiesterase in some tissues (Beavo *et al.*, 1970; Teresaki & Appleman, 1975; Adachi *et al.*, 1976), but in the guinea-pig pancreas cyclic GMP concentrations of up to 10 μM had no significant effect when the cyclic AMP concentration was 5 μM . Cyclic GMP phosphodiesterase was inhibited by cyclic AMP ($17 \pm 6\%$, $30 \pm 2\%$ and $54 \pm 3\%$ respectively by 0.1 μM -, 1 μM - and 10 μM -cyclic AMP, at a cyclic GMP concentration of 5 μM). Whether this inhibition has any physiological significance is not known.

Table 1. Subcellular distribution of cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase in the guinea-pig pancreas

Guinea-pig pancreas was homogenized in either Tris/sucrose buffer as described in the Methods section or Tris/sucrose buffer containing 0.5% (w/v) Triton X-100. The specific activities in the fractions are means $\pm$ S.E.M. for triplicate determinations, with the total activity expressed as percentage of the homogenate activity given in parentheses. The lactate dehydrogenase activity in each fraction is also given as percentage of the activity in the homogenate.

	Presence of Triton X-100 ...	Cyclic AMP phosphodiesterase activity (pmol of cyclic AMP/min per mg of protein)		Cyclic GMP phosphodiesterase activity (pmol of cyclic GMP/min per mg of protein)		Lactate dehydrogenase activity (%)	
		—	+	—	+	—	+
Homogenate		224 $\pm$ 17 (100)	158 $\pm$ 19 (100)	125 $\pm$ 14 (100)	121 $\pm$ 10 (100)	100	100
10 min $\times$ 2000 g pellet (P1)		158 $\pm$ 8 (11.4)	96 $\pm$ 14 (3.9)	64 $\pm$ 5 (8.7)	66 $\pm$ 5 (3.5)	3.3	1.6
30 min $\times$ 30 000 g pellet (P2)		112 $\pm$ 2 (8.7)	38 $\pm$ 1 (1.4)	23 $\pm$ 5 (3.4)	29 $\pm$ 1 (1.4)	2.1	1.6
60 min $\times$ 300 000 g pellet (P3)		91 $\pm$ 3 (1.9)	100 $\pm$ 4 (2.4)	39 $\pm$ 3 (1.5)	49 $\pm$ 4 (1.5)	2.1	1.0
60 min $\times$ 300 000 g supernatant		383 $\pm$ 21 (40.9)	144 $\pm$ 26 (35.2)	324 $\pm$ 3 (65.7)	151 $\pm$ 8 (30.9)	75.2	84.4
Total recovered activity		(62.9)	(42.9)	(79.3)	(37.3)	82.7	89.6

Fig. 1. *Effect of metal cation chelators on cyclic nucleotide phosphodiesterase activity*

Cyclic AMP phosphodiesterase activity (a) and cyclic GMP phosphodiesterase activity (b) were measured in the supernatant from guinea-pig pancreas in the presence of EDTA (O) and EGTA (●). Results are means $\pm$ S.E.M. for triplicate determinations.

Fig. 2. *Effect of Mg^{2+} on cyclic nucleotide phosphodiesterase activity*

Cyclic AMP phosphodiesterase activity (a) and cyclic GMP phosphodiesterase activity (b) were measured in the absence of added cations (●), and in the presence (O) or absence (●) of 1 mM- Ca^{2+} , at various concentrations of Mg^{2+} . The ion-free supernatant from guinea-pig pancreas was used as the source of enzyme. Results are expressed as means $\pm$ S.E.M. for triplicate determinations.

Inhibition of cyclic nucleotide phosphodiesterases by methylxanthines and papaverine

Since methylxanthines and papaverine are often used *in vivo* and in intact-cell experiments *in vitro*

as inhibitors of cyclic nucleotide phosphodiesterase activity, their potencies in broken-cell preparations are important in interpreting the data obtained. However, further factors, such as cell penetration

Fig. 3. Effect of Ca^{2+} on cyclic nucleotide phosphodiesterase activity

Cyclic AMP phosphodiesterase (a) and cyclic GMP phosphodiesterase (b) were measured in the ion-free supernatant from guinea-pig pancreas (prepared as described in the Methods section) in the absence of any added cations ($\diamond$), or in the presence of 3 mM- Mg^{2+} (O) or 3 mM- Mn^{2+} ($\bullet$), at increasing concentrations of Ca^{2+} ($\blacksquare$). Results are expressed as means $\pm$ S.E.M. for triplicate determinations.

and effects other than on cyclic nucleotide phosphodiesterases (Breckenridge, 1970; Schwabe & Ebert, 1972), must be taken into account. The I_{50} concentrations for cyclic AMP were 21 μM (papaverine), 63 μM (isobutylmethylxanthine), 47 μM (theophylline) and 1.9 mM (caffeine). For cyclic GMP phosphodiesterase the I_{50} concentrations were 12 μM (papaverine), 15 μM (isobutylmethylxanthine), 2.5 mM (theophylline) and 3.2 mM (caffeine).

Inactivation of cyclic nucleotide phosphodiesterase on storage

Cyclic GMP phosphodiesterase was markedly more labile than cyclic AMP phosphodiesterase on storage. Both enzymes were completely inactive after 1 week at room temperature, but, whereas only 30% of cyclic AMP phosphodiesterase activity was lost after 1 week at 4°C, cyclic GMP phosphodiesterase was completely inactivated under these conditions (Table 2). The pancreas contains powerful proteolytic enzyme precursors (zymogens), which can be activated by autocatalytic mechanisms (Bhoola & Dorey, 1971). It is possible that such proteolytic enzymes may contribute to the lability of cyclic nucleotide phosphodiesterase activity during storage, and hence the effect of Trasylol, which inhibits trypsin-like proteolytic enzymes, was measured. Trasylol itself had no effect on cyclic nucleotide phosphodiesterase activity. Cyclic GMP phosphodiesterase was

partially protected during storage by Trasylol added at the time of homogenization, suggesting that activated zymogens may indeed contribute to the instability of this enzyme. Cyclic AMP phosphodiesterase exhibited a dual response, which may be explained by the finding that cyclic AMP phosphodiesterase can be activated by trypsin (Thompson & Strada, 1978). The increase in cyclic AMP phosphodiesterase activity occurring after storage for 2 days at 4°C could be explained by limited proteolytic activity, and Trasylol added at the time of homogenization inhibited this increase. More extensive proteolysis, such as would be expected after storage for 2 days at 20°C, would produce a net decrease in cyclic AMP phosphodiesterase activity; under these conditions, Trasylol present during storage had a protective effect.

Action on physiologically active substances

Cholecystokinin-pancreozymin, secretin and carbachol (all tested at 20 nM and at 1 μM) were without any significant effect on the cyclic AMP phosphodiesterase or cyclic GMP phosphodiesterase activity of either the 300 000 g supernatant or 300 000 g pellet fractions.

Kinetic parameters

Plots of the reciprocal of cyclic nucleotide concentration against reciprocal of activity for both cyclic AMP phosphodiesterase and cyclic GMP

Table 2. *Stability of cyclic nucleotide phosphodiesterase activity during storage*

Activity (pmol/min per mg of protein)				
Enzyme	Temperature (°C)	Length of storage (days)		
		...	0	7
Cyclic AMP phosphodiesterase	-20	...	-	-
	+4	...	49.1 ± 2.1	36.4 ± 1.7
	+20	...	48.2 ± 1.1	23.2 ± 1.4
Cyclic GMP phosphodiesterase	-20	...	-	-
	+4	...	26.1 ± 1.1	34.5 ± 1.7
	+20	...	24.6 ± 1.3	2.3 ± 1.4

phosphodiesterase suggested the presence of a high- and a low-affinity enzyme (Fig. 4). Each component was analysed by linear-regression analysis (Wilkinson, 1961). The respective K_m values of cyclic AMP phosphodiesterase were $6.5 \pm 1.1 \mu\text{M}$ (substrate concentration $2.5\text{--}10 \mu\text{M}$) and $0.56 \pm 0.05 \text{ mM}$ (substrate concentration $10\text{--}1 \text{ mM}$), and of cyclic GMP phosphodiesterase $31.9 \mu\text{M}$ (substrate concentration $3\text{--}100 \mu\text{M}$) and $0.32 \pm 0.03 \text{ mM}$ (substrate concentration $100\text{--}1000 \mu\text{M}$). These multiple affinities are probably due to the presence of several molecular forms, as was first suggested by Thompson & Appleman (1971).

Separation of cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase by chromatography

The presence of more than one molecular form of cyclic nucleotide phosphodiesterase was verified by partial purification as described in the Methods section. The initial gel-filtration step did not result in any separation of the activities, but removed most of the inactive from the active material. The ion-

Fig. 4. *Plots of the reciprocals of soluble cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase activities against the reciprocal of substrate concentration* Cyclic AMP phosphodiesterase (a) and cyclic GMP phosphodiesterase (b) activities in the 300 000 g supernatant fraction of guinea-pig pancreas were measured at substrate (cyclic AMP or cyclic GMP) concentrations ranging from 2.5 to $1000 \mu\text{M}$. Each value is the mean of triplicate determinations.

Fig. 5. Separation of cyclic AMP phosphodiesterase from cyclic GMP phosphodiesterase activity on DEAE-Sephadex

The cyclic AMP-hydrolysing (●) and cyclic GMP-hydrolysing (○) activities were measured in 50 µl samples taken from the fractions eluted from DEAE-Sephadex as described in the Methods section. The concentration of KCl in the fractions (—) was measured conductimetrically. Fraction size was 18 ml.

exchange chromatography, however, resulted in separation of a cyclic AMP-specific peak from a cyclic GMP-specific peak (Fig. 5). The early-eluted shoulder of the cyclic AMP-specific peak overlapped with a second peak of cyclic GMP-hydrolysing activity, suggesting the presence of a poorly resolved third component that was not specific for either cyclic nucleotide.

Discussion

The subcellular distribution of both cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase in the guinea-pig pancreas suggested a predominantly cytoplasmic location. As in the rat pancreas (Rutten *et al.*, 1973), the cyclic AMP phosphodiesterase activity in the pellet fractions was greater than could be accounted for by cytoplasmic trapping, indicating the presence of a distinct particulate enzyme. On the other hand, most of the activity of cyclic GMP phosphodiesterase in the pellet fractions could be attributed to trapped cytoplasm, and, if there was a true particulate component, it represented only a very small proportion of the total activity. These pellet-associated

enzymes were not characterized in detail, but it was established that pancreatic secretogens had no direct effect on the activity of the cyclic phosphodiesterases.

In guinea-pig pancreas, as in most tissues examined, multiple cyclic nucleotide phosphodiesterase activity was observed. Both the cyclic AMP- and cyclic GMP-hydrolysing activities had high- and low-affinity components. Since mean tissue concentrations of cyclic AMP and cyclic GMP in the guinea-pig pancreas remain below 1 µM even during stimulation (estimated from the data given by Albano *et al.*, 1976), the contribution of the low-affinity enzymes would therefore be negligible, unless a compartmentation of cyclic nucleotides gave rise to local concentrations very much in excess of the mean tissue value. For this reason we used in our assay a concentration of cyclic nucleotide substrate of 5 µM, at which the activity of the high-affinity component could be readily measured while excluding the contribution of the low-affinity component.

The effects of Ca^{2+} and Mg^{2+} on the activity of supernatant cyclic nucleotide phosphodiesterases were clearly interdependent. In the presence of 3 mM- Mg^{2+} , which was close to the maximally stimulating concentration, the addition of 1 mM- Ca^{2+} had very little effect. If, however, the concentration of Mg^{2+} was decreased to below 1 mM, both cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase activities became markedly dependent on the presence of Ca^{2+} . A Ca^{2+} -dependent protein activator (calmodulin) appears to offer a likely mechanism for this activation (Larsen & Vincenzi, 1979). Christophe *et al.* (1977) have reported the presence of such a protein in the rat pancreas, but it was specific to cyclic GMP phosphodiesterase. Ca^{2+} -dependent regulator proteins have been reported for cyclic AMP phosphodiesterases in some tissues (Smoake *et al.*, 1974; Kakiuchi *et al.*, 1975), but not so far in the pancreas.

The supernatant cyclic nucleotide-hydrolysing activity could be clearly separated by chromatography on DEAE-Sephadex into a cyclic GMP-specific and a cyclic AMP-specific peak. There was suggestion of a further component, poorly resolved from the cyclic AMP-specific peak, that hydrolysed both cyclic nucleotides at the same rate. This pattern is similar to that reported in the rat pancreas (Terai *et al.*, 1976; Vandermeers *et al.*, 1977).

Functional considerations

The heterogeneity of exocrine pancreatic tissue must be considered in attempting to interpret these results. The bulk of the exocrine pancreas comprises acinar cells to which is attributed the function of secreting proteins in response to stimulation by acetylcholine or cholecystokinin-pancreozymin (see

Thomas, 1967). Most of the remainder of the tissue consists of centroacinar and duct cells, which secrete bicarbonate and electrolyte components of pancreatic fluid in response to stimulation by secretin. This functional and morphological dichotomy raises the question whether the properties of the cyclic nucleotide phosphodiesterases are also different in the protein- and electrolyte-secreting cells; this uncertainty will not be resolved until studies have been carried out with homogeneous cell preparations.

Activation of guanylate cyclase remains the most likely explanation for the initial rise in tissue cyclic GMP concentrations observed during incubation of pancreatic tissue with acetylcholine or cholecystokinin-pancreozymin (Albano *et al.*, 1976). At concentrations of cellular Mg^{2+} below 1 mM, an associated increase in intracellular Ca^{2+} would produce an increase in the activity of cyclic GMP phosphodiesterase. This may be the reason for the rapid return of cyclic GMP concentrations to basal values. There is indirect experimental support for this in that if cyclic GMP concentrations are raised in the absence of Ca^{2+} , by incubating pancreatic acinar cells with sodium nitroprusside, the concentrations remain raised and do not return to basal values (Lopatin & Gardner, 1978). A similar Ca^{2+} -induced activation of cyclic AMP phosphodiesterase when acinar cells are stimulated provides a possible explanation for the observation that cholecystokinin-pancreozymin does not increase cyclic AMP concentrations in the guinea-pig pancreas, despite the fact that it activates adenylate cyclase in this tissue (Lemon *et al.*, 1979). The rise in cyclic AMP concentrations in response to secretin (Albano *et al.*, 1976) probably occurs in the duct and centroacinar rather than in the acinar cells. Cyclic AMP phosphodiesterase activity could be modulated by Ca^{2+} , as described for acinar cells, but, since the ion changes that take place in these cells have not yet been established, the form that this modulation would take cannot be predicted.

P.M. was a Medical Research Council Scholar and M.L. a Wellcome Trust Fellow. K.B. gratefully acknowledges generous financial support from the Wellcome Trust and the Medical Research Council and thanks Smith, Kline and French for an equipment grant.

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