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Butyryl Derivatives of Cyclic GMP Interfere with the Biological and the Immunological Properties of the Pancreozymin–Gastrin Family of Peptides

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SUMMARY

ROBBERECHT, P., M. DESCHODT-LANCKMAN, M. -C. WOUSSEN-COLLE, P. DE NEEF, J. CAMUS AND J. CHRISTOPHE. Butyryl derivatives of cyclic GMP interfere with the biological and the immunological properties of the pancreozymin–gastrin family of peptides. *Mol. Pharmacol.* 17: 268–274 (1980).

A comparison has been made of the ability of dibutyryl cyclic GMP and parent nucleotides to compete with ^{125}I-pancreozymin for binding to rat pancreatic plasma membranes. A parallel study of the inhibitory effects of these nucleotides on the stimulation by the C-terminal octapeptide of pancreozymin on (a) amylase secretion from pancreatic fragments, and (b) adenylate cyclase activity in pancreatic plasma membranes has been conducted. The data suggest a specific, immediate, and full competitive inhibition exerted by dibutyryl cyclic GMP on the binding of peptides of the pancreozymin family (including tetragastrin) to their specific receptors. The presence of a butyryl side chain on the purine base was essential for inhibiting hormone binding and the resulting activation of adenylate cyclase. In addition, the second butyryl side chain on the ribose moiety allowed maximum efficacy. The effects of dibutyryl cyclic GMP did not bear directly on the catalytic subunit of adenylate cyclase, and did not depend on the presence of nucleotide triphosphates. The fast dissociation states evoked by a high degree of occupancy of hormone receptors by pancreozymin analog or by the addition of GTP were not reproduced by dibutyryl cyclic GMP. In general, the biological potencies of dibutyryl cyclic GMP analogs on the exocrine pancreas were proportional to their capacity to be recognized by pancreozymin–gastrin antibodies.

INTRODUCTION

While studying the combined effects of cyclic nucleotides and peptide hormones on pancreatic secretion, Peikin and Gardner (1) discovered that dibutyryl cyclic GMP (dBcGMP)[1] competitively and specifically inhibits the effect of pancreozymin and its analogs on both enzyme secretion and calcium efflux from isolated guinea pig acini. The authors inferred that the inhibition by the nucleotide takes place at the membrane receptor level.

In the present study we have examined the effects of butyryl nucleotides on pancreozymin stimulation of amylase released from rat pancreatic fragments. Moreover, we examined directly the interaction of butyryl nucleotides on ^{125}I-pancreozymin binding and adenylate cyclase activation in rat pancreatic plasma membranes. Our findings definitely support the conclusion that butyryl derivatives of cyclic GMP inhibit the pancreatic action of pancreozymin and related peptides by virtue of their ability to interact with pancreozymin receptors.

Since structural similarities between pancreozymin-like peptides and butyryl derivatives of cyclic GMP might be responsible for this phenomenon, we studied the interaction of these nucleotides with other pancreozymin "receptors": the antibodies directed against gastrin–pancreozymin-like peptides. We found that the nucleotides were recognized by these antibodies with a relative affinity broadly comparable to that observed with pancreozymin receptors in the rat pancreas.

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[1] The abbreviations used are: dBcGMP, N^2,$O^{2'}$-dibutyryl guanosine 3′:5′-monophosphate; Gpp(NH)p, guanosine (β,γ-imido) triphosphate; OC-PZ, C-terminal octapeptide of pancreozymin; tetragastrin, C-terminal tetrapeptide of pancreozymin and gastrin; EGTA, ethyleneglycol-bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

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MATERIALS AND METHODS

1. *Incubation procedure of pancreas fragments and determination of amylase secretion.* Rat pancreas, trimmed of fat and major blood vessels, were cut into 20- to 30-mg fragments. Four randomly sampled fragments were shaken in 10-ml capacity beakers containing 2 ml of Krebs–Ringer bicarbonate buffer enriched with 10 mM glucose. The pH 7.4 and the oxygenation were maintained under a O_2/CO_2 (95/5, v/v) atmosphere at 37° in a Dubnoff metabolic shaker. After a 10-min preincubation period, the medium was replaced by fresh buffer containing the C-terminal octapeptide of pancreozymin (OC-PZ), tetragastrin and dBcGMP as specified in the legend to Fig. 1. After 30 min, the incubation was terminated by pipetting the incubation medium. Amylase activity in the medium was determined using the saccharogenic method of Noelting and Bernfeld (2) as automated by Vandermeers *et al.* (3). The unit of amylase was defined as the amount of enzyme that liberates a reducing power equivalent to 1 μmole of maltose/min at 25° and pancreatic amylase secretion was expressed in units per 100 mg tissue/30 min.

2. *Adenylate cyclase assay.* Rat pancreatic adenylate cyclase was estimated on a stable hormone-responsive plasma membrane preparation (4) by measuring the amount of cyclic [^{32}P]AMP formed from [α-^{32}P]ATP. The assay medium contained 30 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$. 0.5 mM EGTA, 1 mM theophylline, 83 kallikrein inhibitor units/ml of Trasylol, 0.13 mM 2-mercaptoethanol, 1 mM cyclic AMP, 0.5 mM [α-^{32}P]ATP and an ATP regenerating system consisting of 10 mM phospho(enol)pyruvate and 30 μg/ml pyruvate kinase. The reaction was initiated by the addition of 0.01 ml of the membrane preparation and the total incubation volume was 0.06 ml. Under the standard assay conditions, the reaction was terminated after 7 min incubation at 37° by adding 0.5 ml of a 2% sodium dodecyl sulfate solution containing 1 mM ATP and 0.5 mM cyclic [8-^{3}H]AMP (for the determination of cyclic AMP recovery). The separation of cyclic AMP from ATP was achieved by the procedure of Salomon *et al.* (5) with the use of Dowex 50 W-X4 and neutral alumina columns. Each value was determined in duplicate.

3. *Preparation of pancreatic membranes with permanently activated adenylate cyclase.* The detailed procedure was described previously (6). Briefly, rat pancreatic membranes (1–2 mg protein) were incubated at 30° in the combined presence of 30 μM Gpp(NH)p and 0.3 μM OC-PZ in a medium containing 50 mM Tris–HCl buffer (pH 7.4), 5 mM $MgCl_2$, 5 mM dithioerythritol, and mixed hepatic phospholipids (0.2 mg/ml). The incubation was stopped by adding 7 ml of ice-cold buffer A composed of 20 mM Hepes–Tris (pH 7.4) with 0.1 mM $MgCl_2$, 30 mM NaCl, and 0.1 mM dithioerythritol. After immediate centrifugation at 50,000*g* for 5 min at 0°, the pellet was rehomogenized in buffer A and centrifuged again. This washing procedure was repeated three times. The washed membranes were finally resuspended by sonication (3 × 0.5 slc) in buffer A and assayed for adenylate cyclase activity.

4. *Radioiodination of pancreozymin and binding of the tracer to rat pancreatic plasma membranes.* Highly purified porcine pancreozymin was labeled by conjugating ^{125}I-hydroxyphenylpropionic succinimide ester by the method of Bolton and Hunter (7) as described by Rehfeld (8). The labeled peptide was purified by gel filtration (8) then stored at −20° until use within 2 weeks of the radioiodination. The binding of ^{125}I-pancreozymin (30,000 cpm/assay) to rat pancreatic membranes was conducted in a 20 mM Tris–HCl buffer (pH 7.0) containing 0.2% bovine serum albumin, Trasylol (500 kallikrein inactivator units/ml), and 0.5 mg/ml bacitracin in a final volume of 0.12 ml. The reaction was initiated by the addition of 20 to 30 μg plasma membrane protein and conducted at 37°. Separation of membrane-bound and free hormone was achieved by adding 2 ml of ice-cold 0.1 M sodium phosphate buffer (pH 7.2) enriched with 0.2% albumin followed by immediate filtration of the mixture through EHWP cellulose acetate filters of 0.45-μm pore size (Millipore Corp.). The radioactivity remaining on the filters was counted in a gamma spectrometer. All assays were performed in duplicate and in each experiment the nonspecific binding of the tracer was determined by incubating the membranes and the tracer in the presence of 2 μM caerulein, an analog of pancreozymin.

5. *Gastrin radioimmunoassay.* Gastrin radioimmunoassay was conducted as previously described using rabbit antibody 3737 b of known specificity (9). Bound and free tracer were separated with charcoal coated with human serum. Synthetic human gastrin I was labeled using the chloramine-T method (10). The monoiodinated form, prepared according to Brown *et al.* (11), was used as the radioactive ligand. Synthetic human gastrin I was used as a standard and the results are expressed as picogram equivalents of this standard.

6. *Origin of peptides and chemicals.* Natural porcine secretin and pancreozymin–cholecystokinin were generous gifts from Dr. Viktor Mutt (Karolinska Institutet, Stockholm, Sweden). Bombesin and caerulein were gifts from Dr. Roberto de Castiglione (Farmitalia, Montedi-

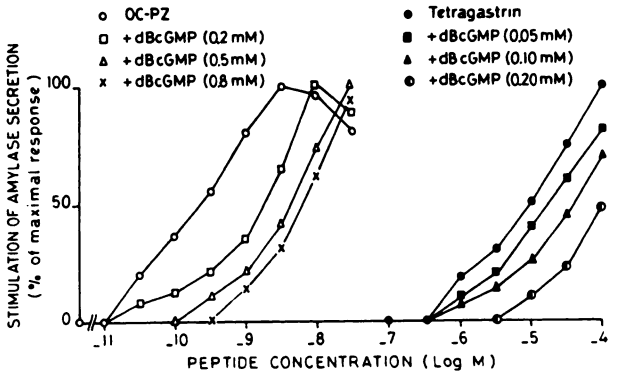

FIG. 1. *Dose–effect curves of C-terminal octapeptide of pancreozymin (OP-PZ: open symbols) and C-terminal tetrapeptide of pancreozymin (tetragastrin: darkened symbols) on amylase secretion from rat pancreatic fragments*

Incubations were conducted in the absence (○, ●) or in the presence of 0.05 mM (■), 0.1 mM (▲), 0.2 mM (□, ◐), 0.5 mM (△), and 0.8 mM (▽) dBcGMP. The results were expressed as a percentage of maximum amylase secretion over basal secretion observed in the presence of 3×10^{-9} M OC-PZ and represent the means of three experiments.

son, Milan, Italy). The C-terminal octapeptide of pancreozymin (OC-PZ) was a gift from the Squibb Institute for Medical Research (Princeton, N.J.). Tetragastrin and synthetic human gastrin I were purchased, respectively, from Senn Chemical (Dielsdorf, Switzerland) and ICI (Macclesfield, England). Trasylol was a gift from Bayer Pharma (Brussels, Belgium).

All nucleotides were obtained from Sigma Chemical Company (St. Louis, Mo.) and ATP was purified before use by the method of Kimura and Nagata (12). The labeled compounds ([α-^{32}P]ATP, cyclic [8-^{3}H]AMP, ^{125}I-hydroxyphenylpropionic succinimid ester, and ^{125}INa) were purchased from the Radiochemical Centre (Amersham, England). All other chemicals were of the highest grade available.

RESULTS

Effect of dibutyryl cyclic GMP on amylase secretion from rat pancreatic fragments. Dibutyryl cyclic GMP (0.05 to 1.0 mM dBcGMP) had no effect on basal and on bombesin- or carbamylcholine-stimulated amylase release (data not shown). In contrast, the nucleotide inhibited the increase in enzyme secretion caused by the OC-PZ or by the C-terminal tetrapeptide of pancreozymin and gastrin (tetragastrin) (Fig. 1). The nucleotide did not alter the maximal increase in amylase secretion caused by OC-PZ. The competitive character of the inhibition was evident from the parallel rightward shift in the dose–effect curve for OC-PZ-stimulated amylase secretion. Although tetragastrin concentrations above 10^{-4} M were not tested, a similar competition was likely from the displacement of dose–effect curves of tetragastrin. Assuming competitive inhibition, an apparent inhibitory constant of 0.04 mM for dBcGMP was calculated irrespective of the agonist tested (13).

Effect of dibutyryl cyclic GMP on adenylate cyclase stimulation. Dibutyryl cyclic GMP at concentrations as high as 2 mM did not alter the basal, secretin- and guanyl

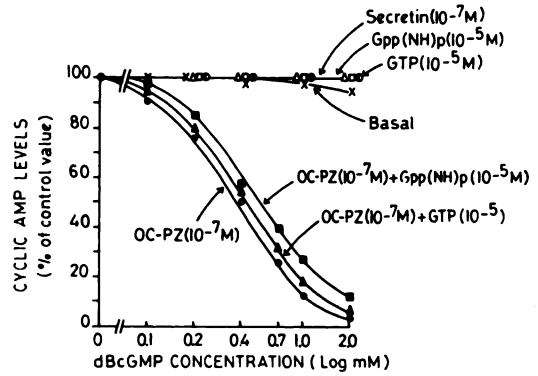

FIG. 2. *Dose–effect curve of inhibition of rat pancreatic plasma membrane adenylate cyclase activity by dBcGMP in the absence of any stimulus* (×) *or in the presence of* 10^{-5} M *GTP* (△), 10^{-5} M *Gpp(NH)p* (□), 10^{-7} M *secretin and* 10^{-5} M *GTP* (◐), 10^{-7} M *OC-PZ* (●), 10^{-7} M *OC-PZ and* 10^{-5} M *GTP* (▲), *and* 10^{-7} M *OC-PZ and* 10^{-5} M *Gpp(NH)p* (■)

The absolute values of the effects of the stimuli were similar to those previously published (14, 28). The adenylate cyclase activity was determined as described under Materials and Methods. Results were the means of three to six experiments and were expressed as percentage of cyclic AMP formed in the absence of dBcGMP (control value).

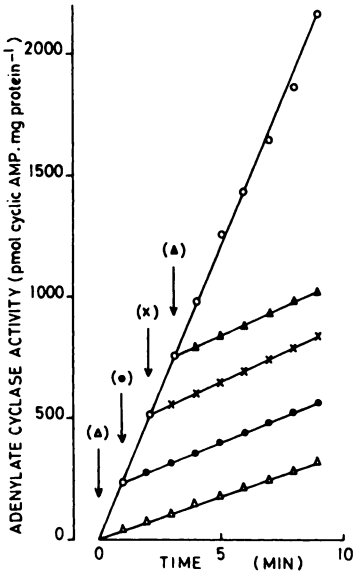

FIG. 3. *Time course of inhibition of OC-PZ-stimulated adenylate cyclase by 1 mM dBcGMP*

Adenylate cyclase activity was measured at 37° in the presence of 10^{-7} M OC-PZ and 10^{-5} M GTP. The reaction was started by addition of the membranes and dBcGMP was added at time 0 (△), 1 min (●), 2 min (×) or 3 min (▲) as indicated by the arrows. This experiment is typical of two others.

nucleotide-stimulated adenylate cyclase activities, but abolished the increase in adenylate cyclase activity caused by OC-PZ (Fig. 2). At the submaximal OC-PZ concentration of 10^{-7} M (14), the inhibition was already detected with 0.1 mM dBcGMP and was almost complete at 2.0 mM dBcGMP. The addition of 10^{-5} M GTP or 10^{-5} M Gpp(NH)p did not alter the inhibitory effects of dBcGMP.

In the following experiments, the plasma membrane preparation was incubated in the combined presence of 10^{-7} M OC-PZ and 10^{-5} M GTP in order to stimulate adenylate cyclase activity maximally (14), and the time course of the inhibitory effects of dBcGMP was investigated. In control experiments, the adenylate cyclase activity was linear in the presence of 10^{-7} M OC-PZ and 10^{-5} M GTP. The inhibition by dBcGMP was immediate and independent of the time of addition (Fig. 3).

In the presence of increasing concentrations of dBcGMP, the dose–response curve of OC-PZ-stimulated adenylate cyclase was shifted to the right, suggesting that the inhibition was competitive (Fig. 4). The apparent K_i calculated as reported in Fig. 1 was around 0.08 mM. Similar values were observed when adenylate cyclase was stimulated by natural porcine pancreozymin or by caerulein (data not shown). Butyrate, cGMP and 5′-GMP when tested at concentrations as high as 7 mM exerted no effect on adenylate cyclase activity (data not shown).

Effect of dibutyryl cyclic GMP on persistently activated adenylate cyclase. Pancreatic membrane adenylate cyclase was maximally activated by a preincubation in the presence of both OC-PZ and the stable nucleotide Gpp(NH)p. The persistently active state observed after extensive washing of the membranes was not inhibited by dBcGMP (Fig. 5).

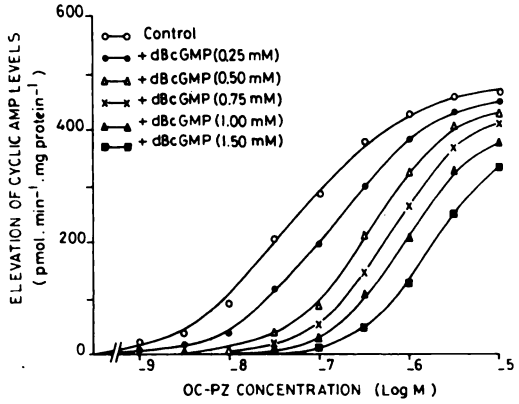

FIG. 4. *Inhibitory effects of dBcGMP on the dose–effect curve of activation of adenylate cyclase by OC-PZ*

Cyclic AMP produced was determined after 7 min incubation at 37° in the absence (○) or in the presence of 0.25 mM (●), 0.50 mM (△), 0.75 mM (×), 1.0 mM (▲), and 1.5 mM (■) dBcGMP. The results were expressed as pmole cyclic AMP min^{-1} mg membrane protein^{-1} produced in excess of the basal unstimulated value. The curves were the means of three experiments performed in duplicate.

Effect of various nucleotides on adenylate cyclase activity. Cyclic GMP, 8-bromo-cyclic GMP, 5′-GMP, butyrate, cyclic AMP, and 5′-AMP did not inhibit (not shown) whereas butyryl derivatives of cyclic nucleotides did inhibit the increase in adenylate cyclase activity caused by OC-PZ to various extents (Fig. 6). The three cyclic nucleotides monobutyrylated on the ribose moiety ($O^{2'}$-mBcGMP, $O^{2'}$-mBcGMP and $O^{2'}$-mBcIMP) were 10–20-fold less potent than dBcGMP in inhibiting this stimulation by OC-PZ. Dibutyryl cyclic AMP was less active and N^2-mBcGMP and N^6-mBcAMP, two cyclic nucleotides monobutyrylated on the purine base, were even less potent.

Effect of dibutyryl cyclic GMP on ^{125}I-pancreozymin binding to pancreatic plasma membranes. The properties and characteristics of the binding of ^{125}I-pancreozymin to rat pancreatic plasma membranes were similar to those previously reported for the binding of [^{3}H]caerulein on the same preparation (15, 16). OC-PZ and a series of pancreozymin-like peptides, including tetragastrin as well as dBcGMP, inhibited the binding of the tracer molecule. The concentrations required for half-maximal inhibition of ^{125}I-pancreozymin binding were 2.5×10^{-8} M for pancreozymin, 3×10^{-5} M for tetragastrin, and 1×10^{-4} M for dBcGMP (Fig. 7).

Effect of various nucleotides on ^{125}I-pancreozymin binding to pancreatic plasma membranes. Cyclic nucleotides and butyryl cyclic nucleotides were tested for their ability to inhibit the binding of ^{125}I-pancreozymin to pancreatic plasma membranes (Fig. 8). These results were similar to those observed for the inhibition of OC-PZ-stimulated adenylate cyclase. Dibutyryl cyclic AMP and the *O*-monobutyryl derivatives of cyclic GMP, cyclic AMP, and cyclic IMP were equally potent but were approximately 20-fold less potent than dBcGMP in inhibiting the binding of ^{125}I-pancreozymin. The *N*-monobutyryl derivative of cyclic GMP was even less potent and N^6-mBcAMP did not alter ^{125}I-pancreozymin binding. Butyrate was inactive.

Effect of dBcGMP on the dissociation of ^{125}I-pancreozymin bound to pancreatic plasma membranes. We reported previously that guanyl nucleotides (17) and pancreozymin-like peptides (15, 17) increase the rate of dissociation of membrane bound [^{3}H]caerulein from rat pancreatic plasma membranes. These reagents were also found to increase the rate of dissociation of ^{125}I-pancreozymin from the same plasma membranes (Table 1). Dibutyryl cyclic GMP did not significantly alter pancreozymin dissociation, and did not modify the effect of GTP on the tracer dissociation. In contrast, dBcGMP did inhibit the capacity of unlabeled caerulein to promote the dissociation of ^{125}I-pancreozymin (Table 1).

Interference of nucleotides with the gastrin radioimmunoassay. A series of cyclic and noncyclic nucleotides were tested for their ability to inhibit the reversible binding of monoiodinated human gastrin I to rabbit gastrin antibodies. When inhibition occurred, the concentration of nucleotide was expressed in human gastrin I equivalents (Table 2). The gastrin antibodies recognized all the butyryl nucleotides as well as cyclic GMP. Several other nucleotides as well as butyrate were not recognized by the gastrin antibodies. Dose–effect curves for the butyryl nucleotides were parallel to the standard gastrin curve and dBcGMP caused no inhibition of binding of free gastrin I to charcoal (data not shown).

DISCUSSION

The ability of dBcGMP to inhibit pancreozymin-stimulated amylase secretion from rat pancreatic fragments (Fig. 1) might conceivably be attributed to either an

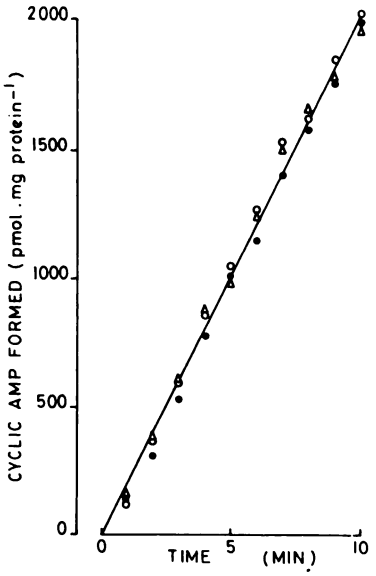

FIG. 5. *Lack of effect of dBcGMP on the time course of activity of permanently activated pancreatic adenylate cyclase*

Pancreatic plasma membranes were activated as described under Materials and Methods and in Ref. (6) by a preincubation in the presence of 10^{-6} M OC-PZ and 10^{-5} M Gpp(NH)p. After extensive washing, the activated membranes were tested in the standard assay medium under three conditions: in the absence of any added agent (○), in the combined presence of 10^{-7} M OC-PZ and 10^{-5} M Gpp(NH)p (●), and in the presence of 1 mM dBcGMP (△). This experiment is representative of two others.

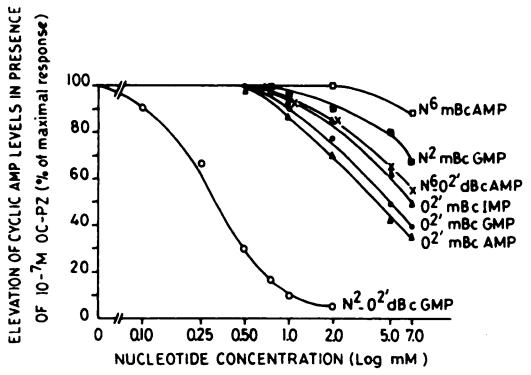

FIG. 6. *Specificity of the inhibition of OC-PZ stimulation of rat pancreatic plasma membrane adenylate cyclase by butyryl derivatives of cyclic nucleotides*

The adenylate cyclase activity was tested as described under Materials and Methods in the presence of 10^{-7} M OC-PZ and increasing concentrations of the following butyryl cyclic nucleotides: dBcGMP (○), $O^{2'}$-mBcAMP (▲), $O^{2'}$-mBcGMP (●), $O^{2'}$-mBcIMP (△), dBcAMP (×), N^2-mBcGMP (■), or N^6-mBcAMP (□). The results were expressed as percentage of the control value observed in the presence of OC-PZ alone and were the means of three to five separate experiments.

interaction between the nucleotide and the peptide, a competition between both agents at the membrane receptor level, or an effect of the nucleotide on the intracellular process leading to emiocytosis. An interaction outside the cell is not definitively excluded but is unlikely considering the acidic character of both compounds and the fact that the apparent inhibitory constant for dBcGMP was identical when OC-PZ and the shorter peptide tetragastrin were used as agonists. An inhibitory effect of dBcGMP on those steps of the stimulus–secretion coupling process following hormone binding was also unlikely as the nucleotide did not modify the effects of bombesin and carbamylcholine which act via the same intracellular mode of action as pancreozymin-like peptides (18, 19).

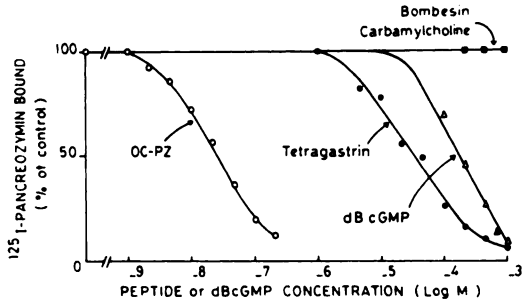

FIG. 7. *Inhibition of ^{125}I-pancreozymin binding to rat pancreatic plasma membranes by OC-PZ* (○), *tetragastrin* (●), *and dBcGMP* (△)

Pancreatic plasma membranes were incubated as described under Materials and Methods with 3×10^{-10} M ^{125}I-pancreozymin in the presence of increasing concentrations of the tested agents. After 15 min incubation at 37° the membrane bound peptide was separated from the free peptide by filtration. In each experiment, the nonspecific binding (including the nonspecific interference of Millipore filters), as determined by incubating membranes and tracer in the presence of 3×10^{-6} M unlabeled caerulein, was subtracted. The results, expressed as percentage of radioactivity bound in presence of tracer only, were the means of three experiments.

The present study on rat pancreatic plasma membranes provides strong evidence that the inhibitory effect of dBcGMP on pancreozymin stimulation took place at the peptide receptor level. The interference of dBcGMP was approached by examining the effect of the nucleotide on hormone-stimulated adenylate cyclase activity and on the binding of a biologically active preparation of ^{125}I-pancreozymin to pancreatic plasma membranes.

Three mechanisms might account for the specific and competitive inhibition of pancreozymin-stimulated adenylate cyclase by dBcGMP: The first is an interaction of the nucleotide with guanyl triphosphate regulatory sites (14, 19) whose occupancy is known to potentiate hormone effects. This possibility was ruled out since dBcGMP did not interfere with the Gpp(NH)p activation of adenylate cyclase and since the inhibitory effect of dBcGMP on pancreozymin-stimulated adenylate cyclase was identical in the absence or in the presence of Gpp(NH)p or GTP. A second possible mechanism is an interaction of dBcGMP with the catalytical subunit of the enzyme complex. This was also excluded as the nucleotide influenced neither the fluoride-stimulated adenylate cyclase activity (data not shown) nor the persistently activated enzyme that exhibits maximal activity in the absence of any added stimulus (6). The third hypothesis of a specific interaction between the nucleotide and pancreozymin receptors at the outer surface of pancreatic plasma membranes was supported by the present data on the binding of ^{125}I-pancreozymin to plasma membranes. Such an interaction might be due to one of three causes: (a) A nonspecific interference of dBcGMP with pancreozymin binding was unlikely as parent nucleotides and free butyrate were markedly less efficient or inefficient even at high concentrations. (b) dBcGMP could interfere with guanyl triphosphate regulatory sites which control in the pancreas (17), as well as in other tissues (20), and affect the binding of the hormone to its receptors. This appears excluded since dBcGMP did not modify the dissociation of the bound peptide (Table 2) and did not influence the increased hormone dissociation evoked by GTP. (c) There was a specific interaction of dBcGMP with pancreozymin receptors. From these considerations it appears that dBcGMP sterically hindered the binding of pancreozymin by reducing the association rate constant

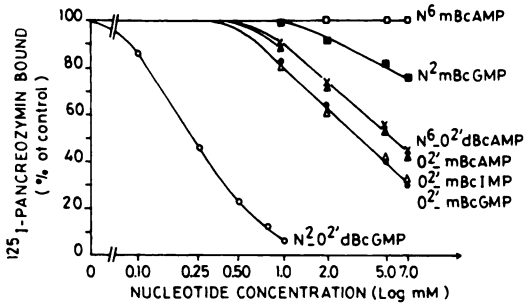

FIG. 8. *Specificity of the inhibitory effects of butyryl derivatives of cyclic nucleotides on the binding of labeled ^{125}I-pancreozymin to rat pancreatic plasma membranes*

The methodology used was identical to that presented in Fig. 7 and the symbols are the same as those presented in Fig. 6.

TABLE 1

Effects of dibutyryl cyclic GMP, GTP and caerulein on the dissociation of ^{125}I-pancreozymin bound to pancreatic plasma membranes

Pancreatic plasma membranes were preincubated for 15 min at 37° in the presence of a tracer concentration of ^{125}I-pancreozymin as described under Materials and Methods and then centrifuged for 8 min at 20,000*g*. The pelleted membranes were resuspended in an equal volume of buffer. Aliquots of 50 μl of the membrane suspension were incubated for 30 min at 37° in 5 ml of a 20 mM Tris–maleate buffer (pH 7.0) containing 0.2% bovine serum albumin, and in the presence or the absence of the tested agent (15). The radioactivity remaining bound to membranes was determined after filtration of the membrane suspension through a 0.45-nm Millipore acetate filter. The results were the mean ± SEM of four experiments.

Additions	Percentage of ^{125}I-pancreozymin remaining bound after a 30-min dissociation period
None	56 ± 5
dBcGMP (7×10^{-4} M)	53 ± 3
GTP (10^{-5} M)	16 ± 2*
Caerulein (3×10^{-8} M)	29 ± 4*
Caerulein + dBcGMP	43 ± 4*,†
Caerulein + GTP	12 ± 2*
dBcGMP + GTP	13 ± 2*

* Values statistically lower ($p < 0.01$) than the control value observed without any agent tested (Student's *t* test on paired values).

† Value statistically higher ($p < 0.01$) than the value observed in presence of caerulein alone.

for hormone binding and not by accelerating the dissociation rate.

The antagonist properties of dBcGMP were not modified by GTP or by Gpp(NH)p, and this characteristic is shared by other antagonists such as atropine (21), β-adrenolytic agents (22), and α-adrenolytic (23). In addition, dBcGMP at a concentration reducing tracer pancreozymin binding (Fig. 7) did not induce an increase of the dissociation rate of pancreozymin bound to plasma membranes (Table 1). In this respect, it is noteworthy that dose–effect curves of dBcGMP developed in a narrower concentration range than those obtained with pancreozymin-like peptides (Figs. 2, 6, 7, and 8). Furthermore, the estimated apparent K_i values of dBcGMP for OC-PZ-stimulation of amylase secretion (4.10^{-5} M, Fig. 1) and adenylate cyclase activity (8.10^{-5} M, Fig. 3) were similar whereas the concentration of OC-PZ required for half-maximal amylase secretion (Fig. 1) was 100 times lower than the apparent K_m of OC-PZ for adenylate cyclase activation (Fig. 4). Thus, unlike the peptide agonist, the binding of dBcGMP to plasma membranes did not cause cooperative interaction of the negative type between pancreozymin receptors (15) perhaps because this antagonist could not induce the efficient coupling with the catalytic subunit of adenylate cyclase (24, 25) which is obtained with the hormone agonist–receptor complex (6, 14).

Gastrin antibodies (9) were used in a parallel study as a tool for studying the interaction of dBcGMP with soluble pancreozymin "receptors." dBcGMP inhibited the binding of labeled gastrin to gastrin antibodies. The parallelism of the dilution curves (data not shown) and the limited number of other nucleotides interfering with the reaction (Table 2) suggest a specific interaction between nucleotides and antibodies rather than nonspecific effects such as those observed with a high 0.2–0.3 M sodium chloride concentration (26). The relative efficacies of various nucleotides in being recognized by gastrin antibodies and pancreatic pancreozymin receptors were comparable. The specificity of the purine determinants for antibody recognition were, however, more stringent, the three monobutyryl derivatives of cyclic GMP being more efficient than the corresponding derivatives of cyclic AMP and cyclic IMP, and cyclic GMP itself being already recognized by gastrin antibodies. From a theoretical point of view, the recognition of dBcGMP by gastrin–pancreozymin antibodies indirectly suggests an analogy between plasma membrane pancreozymin–gastrin receptors and specific soluble immunoglobulins. Such fortuitous analogies have already been postulated (27). This is conceivably due to the heterogeneity of antibody populations: some of the antibodies obtained after immunization might recognize individual determinants in dBcGMP which have their counterpart in features of the peptide hormone that are essential for binding and biological activity. From a more practical point of view, it is important to note that, when applying gastrin–pancreozymin radioimmunoassays to tissue extracts, the presence of nucleotides in large concentrations could interfere with the peptide assays.

In conclusion, the specific recognition of dBcGMP by both pancreatic pancreozymin receptors and gastrin–pancreozymin antibodies suggests the existence of analogies between nucleotide and hormone structures. The minimal amino acid sequence recognized by receptors and antibodies was the tetrapeptide Trp–Met–Asp–Phe–NH_2 whose size is comparable to that of dBcGMP. This finding is reminiscent of the recognition of morphinic agents by endorphin receptors.

TABLE 2

Interference of nucleotides with gastrin radioimmunoassay

"Gastrin" radioimmunoassays were conducted as described under Materials and Methods. All determinations were made in triplicate. The concentrations were expressed as picogram equivalents of the human gastrin I standard.

Nucleotide or fatty acid tested (10 μmol/ml)	Human Gastrin I Equivalents (pg/ml)
$N^2,O^{2'}$-dBcGMP	1165
$O^{2'}$-mBcGMP	669
N^2-mBcGMP	411
3′,5′-cyclic GMP	202
5′-GMP	45*
GDP, GTP	0
$N^6,O^{2'}$-dBcAMP	330
$O^{2'}$-mBcAMP	149
N^6-mBcAMP	216
3′,5′-cyclic AMP	0
5′-AMP	30*
ADP, ATP	0
$O^{2'}$-mBcIMP	251
5′-IMP	0
IDP, ITP	0
Butyrate	0

* Values at the limit of detection of the assay.

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