In dispersed acini from guinea pig pancreas, amylase secretion was increased by Bt&cAMP and 8Br-cAMP, but not by native cyclic AMP. These derivatives of cyclic AMP potentiated the increase in amylase secretion caused by cholecystokinin and cholinergic agents but did not alter the increase in enzyme secretion caused by secretin or vasoactive intestinal peptide. Cyclic GMP did not increase amylase secretion and did not modify the increase in amylase secretion caused by various secretagogues. 8Br-cGMP increased amylase secretion apparently by virtue of its ability to mimic the action of endogenous cyclic AMP. Butyryl derivatives of cyclic GMP did not alter amylase release or the increase in amylase release caused by secretin or vasoactive intestinal peptide, but were reversible, competitive antagonists of the action of cholecystokinin and structurally related peptides. The inhibition was specific for cholecystokinin and related peptides since Bt&cGMP did not alter the increase in amylase secretion caused by other secretagogues (e.g., bombesin, physalaemum, carbachol, or A23187) which have a mode of action similar to that of cholecystokinin. Furthermore, this antagonism of the action of cholecystokinin depended absolutely on the presence of a butyryl moiety and the inhibitory potency of butyryl cyclic GMP depended on the number and position of the butyryl side chains.

Previously we have found that pancreatic secretagogues such as cholecystokinin and cholinergic agents act on pancreatic acinar cells to cause a significant increase in calcium outflux, cellular cyclic GMP, and amylase secretion, but do not alter cellular cyclic AMP (1-9). Secretagogues such as secretin and VIP activate adenylate cyclase, increase cellular cyclic AMP, and stimulate amylase secretion but do not alter calcium outflux or cellular cyclic GMP (2, 4, 7, 9). In the present studies we have examined the effects of exogenous cyclic nucleotides as well as their 8-bromo and butyryl derivatives on amylase secretion from dispersed pancreatic acini.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1794 solely to indicate this fact.

1 The abbreviations used are: VIP, vasoactive intestinal peptide; 8Br-cAMP, 8-bromoadenosine 3':5'-monophosphate; 8Br-cGMP, 8-bromoguanosine 3':5'-monophosphate; Bt&cAMP, N'6,0'2'-dibutyryl adenosine 3':5'-monophosphate; Bt&GMP, N',O'-dibutyryl guanosine 3':5'-monophosphate; Ca2+251-tyrosyl methyl ester, 2'-0-succinyl adenosine 3':5'-monophosphate, 2'-0-succinyl guanosine 3':5'-monophosphate, 2'-0-succinyl guanosine 3':5'-monophosphate, N',O'-dibutyryl guanosine 3':5'-monophosphate, 8-bromoguanosine 3':5'-monophosphate, guanosine 3':5'-monophosphate, N6,0'2'-dibutyryl guanosine 3':5'-monophosphate, and guanosine 3':5'-monophosphate from Sigma Chemical Co., St. Louis, Mo.; N6,0'2'-dibutyryl guanosine 3':5'-monophosphate was also obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind., and from ICN, Cleveland, Ohio. Antisera specific for cyclic AMP or cyclic GMP (preconjugated to a second antibody), 2'-0-succinyl guanosine 3':5'-monophosphate, T4-tyrosyl methyl ester, and 8-bromoguanosine 3':5'-monophosphate were obtained from New England Nuclear Corp., Boston, Mass.; basal media (Eagle) amino acids (100 times concentrated) from Grand Island Biological Co., Grand Island, N. Y.; essential vitamin mixture (100 times concentrated) from Microbiological Associates, Bethesda, Md.; silicone oil (d = 1.09) from Aldrich Chemical Co., Inc., Milwaukee, Wis.; glutamine from Research Plus Laboratories, Inc., Denver, N. J.; bovine plasma albumin (Fraction V) from Armour Pharmaceutical Co., Phoenix, Ariz.; silica gel plates from Eastman-Kodak Co., Rochester, N. Y.; diethylaminoethyl cellulose from Whatman Ltd., Kent, England; and Phadebas Amylase Test from Pharmacia Diagnostica, Piscataway, N. J. Synthetic COOH-terminal fragments of porcine cholecystokinin (heptapeptide, octapeptide, decapeptide, and unsulfated heptapeptide) were gifts from Dr. Miguel A. Ondetti, Squibb Institute for Medical Research, Princeton, N. J. COOH-terminal heptapeptide of porcine cholecystokinin with serine sulfate replacing tyrosine sulfate was a gift from Dr. Miklos Bodanszky, Department of Chemistry, Case Western Reserve University, Cleveland, Ohio. Natural porcine cholecystokinin, natural porcine VIP, and natural porcine secretin were gifts from Dr. Viktor Mutt, Gastrointestinal Hormone Research Unit, Karolinska Institutet, Stockholm, Sweden. Caerulein, bombesin, and physalaemum were gifts from Dr. Roberto de Castiglione, Farmitalia Laboratory, Milan, Italy. A23187
was a gift from Dr. Robert Hamil, Eli Lilly and Co., Indianapolis, Ind.

Unless stated otherwise the standard incubation solution contained
24.5 mM Hepes (pH 7.4), 98 mM NaCl, 6 mM KCl, 9.5 mM Na2HPO4,
5 mM sodium pyruvate, 5 mM sodium fumarate, 5 mM sodium glutamate,
11.5 mM glucose, 2.0 mM CaCl2, 1.0 mM MgCl2, 2 mM glutamine,
0.2% (w/v) albumin, 0.01% (w/v) trypsin inhibitor, 1% (v/v) essential
amino acid mixture, and 1% (v/v) essential vitamin mixture.

Methods—Dispersed acini from guinea pig pancreas were prepared
using the procedure described previously (9) and, unless specified
otherwise, were suspended in standard incubation solution containing
0.5 mM calcium, 5 mM theophylline, and 1% (w/v) albumin.

Amylase secretion from pancreatic acini was determined as de-
scribed previously (7, 9). Acini from the pancreas of one animal were
suspended in 200 ml of incubation solution. Samples of cell suspension
were gassed with 100% O2 and incubated with the appropriate agents
at 37°C. At appropriate times 500 µl of cell suspension was centrifuged
at 10,000 x g for 15 s in a Beckman model 153 microcentrifuge.

Supernatant (200 µl) was added to 500 µl of dilute solution
(0.01 M sodium phosphate, pH 7.8, 0.1% (w/v) albumin, and 0.1% (w/v)
sodium dodecyl sulfate). Duplicate 100-µl samples were assayed for
amylase activity by the method of Ceska et al. (10,11) using the
Phadebas reagent. To assay total amylase activity, 1.5 ml of cell
suspension was added to 15 ml of dilute solution and, after vigorous
agitation, duplicate 100-µl samples were assayed for amylase activity.

Amylase secretion was expressed as the per cent of total amylase
activity which was released into the incubation medium during the
incubation. In each experiment, each value was determined in tripli-
cate and the coefficient of variation for triplicate samples was always
less than 10%.

To measure outflux of 45Ca, acini from the pancreas of one animal
were suspended in 10 ml of incubation solution containing 40 µCi of
45Ca, gassed, and preincubated at 37°C for 60 min (1, 3, 5, 6). At the end of the preincubation the cells were washed twice by alternate
centrifugation and resuspension with 100 volumes of incubation so-
lution containing no ?a and resuspended in 40 to 60 ml of standard
centrifugation and resuspension with 100 volumes of incubation so-
lution containing no ?a and resuspended in 40 to 60 ml of standard

Amylase secretion was expressed as the per cent of total amylase
activity which was released into the incubation medium during the
incubation. In each experiment, each value was determined in tripli-
cate and the coefficient of variation for triplicate samples was always
less than 10%.

RESULTS

Amylase release from dispersed pancreatic acini was in-
creased by 8Br-cAMP, Bt3cAMP and 8Br-cGMP but not by
Bt3cGMP (Fig. 1) or by native cyclic nucleotides (not shown).

With derivatives of cyclic AMP, the rate of amylase secretion
was constant during the initial 40 min of incubation and
decreased progressively thereafter (Fig. 1). With 8Br-cGMP,
the rate of amylase secretion was not altered during the initial
30 min of incubation and then increased progressively during the
subsequent 90 min (Fig. 1). When present at maximally
effective concentrations 8Br-cAMP caused a 7-fold increase
in amylase secretion, Bt3cAMP caused a 5-fold increase, and
8Br-cGMP caused a 3-fold increase (Fig. 2). 8Br-cAMP was a
more potent stimulant of amylase release than was Bt3cAMP
and both derivatives of cyclic AMP were more potent secre-
tagogues than 8Br-cGMP (Fig. 2).

To examine the abilities of derivatives of cyclic nucleotides to modify the increase in amylase release caused by other
secretagogues, acini were preincubated with different cyclic
nucleotides for 2 h at 37°C. At the end of the preincubation,
the rate of amylase release was measured during a 15-min
incubation with no additions, CCK-OP, or secretin. Amylase
release with a cyclic nucleotide derivative plus secretin was
the same as that obtained with secretin alone (Table I).

Similar results were obtained using VIP (10 nm) instead
of secretin (not shown). Derivatives of cyclic AMP as well as
8Br-cGMP potentiated the action of CCK-OP on amylase
secretion (Table I). That is, the increase in amylase secretion

FIG. 1 (left). Time course of amylase
release from dispersed acini incubated
with derivatives of cyclic nucleotides.
The per cent of total amylase present in
the incubation medium was determined
times indicated during incubation
at 37°C. Results shown are means of
duplicate determinations from one ex-
periment. This experiment is representa-
tive of three others.

FIG. 2 (right). Effect of derivatives of cyclic nucleotides on amylase release
from dispersed acini prepared from
guinea pig pancreas. The per cent of total
amylase released into the incuba-
tion medium was determined during a
60-min incubation at 37°C. Results
shown are means of duplicate determi-
nations from one experiment and this
experiment is representative of three
others.
caused by CCK-OP plus one of the cyclic nucleotide derivatives was significantly greater than the sum of the increase caused by each agent alone (Table I). Similar results were obtained using carbachol (30 μM) or bombesin (10 nM) instead of CCK-OP (not shown). In other studies, the time course for the potentiation of the action of CCK-OP by a given cyclic nucleotide derivative corresponded to the time course of the action of the same cyclic nucleotide derivative alone. Bt*CAMP, which did not alter the increase in amylase secretion caused by secretin, abolished the increase in amylase secretion caused by CCK-OP (Table II).

To examine the possibility that 8Br-cGMP was contaminated with 8Br-cAMP, we tested both cyclic nucleotide derivatives for their abilities to inhibit binding of 125I-labeled cyclic nucleotides to cyclic nucleotide-specific antibodies. Results obtained from these studies indicated that the maximal amount of 8Br-cAMP which could be present in the sample of 8Br-cGMP was 0.01%.

The present results obtained with dispersed pancreatic acini differ in several respects from those obtained previously using dispersed, single acinar cells (7). Single acinar cells were prepared by incubating the tissue in a calcium-free medium containing EGTA (7), while the dispersed acini used for the present studies were prepared without using a calcium-free, EGTA-containing medium. To assess the potential influence of this calcium-free incubation on the responsiveness of the preparation to various secretagogues, dispersed acini were prepared in the usual fashion except a 20-min incubation with a calcium-free, EGTA-containing medium was added to the digestion procedure. EGTA treatment caused a significant decrease in the stimulation of amylase secretion caused by VIP or CCK-OP (Table II). Bt*CAMP did not alter amylase in control or EGTA-treated acini, but the nucleotide abolished the action of CCK-OP in both preparations (Table II). Bt*CAMP potentiated the action of VIP in EGTA-treated acini but did not alter the action of VIP in control acini (Table II).

In acini incubated with different concentrations of CCK-OP, amylase secretion increased, became maximal with 0.3 nM CCK-OP and then decreased as the concentration of CCK-OP was increased above 0.3 nM (Fig. 3, left). Bt*CAMP caused a parallel, rightward shift in the dose-response curve for CCK-OP-stimulated amylase secretion and the magnitude of the shift was proportional to the nucleotide concentration (Fig. 3, left). Bt*CAMP did not alter the increase in amylase release caused by a maximally effective concentration of CCK-OP (Fig. 3, left). When acini were incubated with a fixed concentration of CCK-OP and different concentrations of Bt*CAMP, two patterns of amylase secretion were observed depending on the treatment of the incubation medium with 8Br-cGMP.

**TABLE I**

| Incubation | Amylase release after preincubation with
|---|---|
| | Control | 8Br-cAMP (0.2 mM) | Bt*cAMP (1 mM) | 8Br-cGMP (5 mM) | Bt*CAMP (0.5 mM) |
| | % total |
| No additions | 1.6 | 4.3 | 5.3 | 3.3 | 2.3 |
| CCK-OP (0.3 nM) | 13.8 | 26.9 | 24.3 | 30.9 | 2.2 |
| Secretin (0.2 μM) | 7.6 | 7.8 | 9.5 | 7.7 | 8.5 |

**TABLE II**

| Additions | Amylase release |
|---|---|
| | Control acini | EGTA-treated acini |
| | % total |
| None | 4.3 ± 0.8 | 5.1 ± 0.9 |
| 8Br-cAMP (1 mM) | 4.4 ± 0.7 | 5.4 ± 0.5 |
| 8Br-cGMP (5 mM) | 26.4 ± 3.4 | 9.2 ± 0.6 |
| Bt*CAMP plus VIP | 25.9 ± 3.3 | 11.4 ± 1.3 |
| CCK-OP (0.3 nM) | 39.6 ± 4.3 | 11.9 ± 1.2 |
| Bt*CAMP plus CCK-OP | 5.8 ± 1.6 | 4.8 ± 0.6 |

*a Significantly different (p < 0.01) from corresponding value without Bt*CAMP by Student's paired t test.

**FIG. 3.** Effect of Bt*CAMP on the increase in amylase secretion caused by CCK-OP. The per cent of total amylase released into the incubation medium was determined during a 30-min incubation at 37°C. In the left panel the concentrations of Bt*CAMP are given in parentheses. In the right panel the concentrations of CCK-OP are given in parentheses. Results shown are means of triplicate determinations from one experiment and this experiment is representative of five others.
on the concentration of CCK-OP. With submaximal or maximally effective concentrations of CCK-OP, BtzcGMP caused a concentration-dependent decrease in amylase secretion and with higher concentrations of CCK-OP, higher concentrations of BtzcGMP were required to abolish stimulation of amylase secretion (Fig. 3, right). With supramaximal concentrations of CCK-OP, as the concentration of BtzcGMP was increased, amylase secretion increased, became maximal, and then decreased toward basal values (Fig. 3, right). The results illustrated in Fig. 3 were obtained using BtzcGMP from Sigma Chemical Co. Similar results were obtained using BtzcGMP from ICN or from Boehringer Mannheim Biochemicals.

Results similar to those obtained measuring amylase secretion with CCK-OP and BtzcGMP were also obtained with carbachol and atropine (Fig. 4). That is, as the concentration of carbachol was increased, there was a progressive rightward shift in the dose-response curve for atropine inhibition. Furthermore, with concentrations of carbachol which were supramaximal for amylase secretion, as the atropine concentration increased, amylase secretion increased, became maximal and then decreased to basal values (Fig. 4).

In addition to BtzcGMP, monobutryl derivatives of cyclic GMP also inhibited the stimulation of amylase secretion caused by CCK-OP (Fig. 5). O'-Monobutyryl cyclic GMP was approximately 20 times less potent than the dibutyryl derivative while N'-monobutyryl cyclic GMP was approximately 100 times less potent than the dibutyryl derivative (Fig. 5). In addition to native cyclic GMP, O'-tyrosine methyl ester cyclic GMP, O'-succinyl cyclic GMP, and butyrate at concentrations as high as 10 mM did not alter the increase in amylase secretion caused by CCK-OP (not shown).

The inhibitory action of BtzcGMP was specific for cholecystokinin. The nucleotide inhibited the increase in amylase secretion caused by native cholecystokinin, by COOH-terminal heptapeptide, octapeptide, and decapptide of cholecystokinin, by analogues of the COOH-terminal decapptide of cholecystokinin, and by caerulein, a decapptide in which seven of the eight COOH-terminal amino acids are identical with those in the COOH-terminal octapeptide of cholecystokinin (Table III). BtzcGMP did not alter the increase in amylase secretion caused by bombesin, physalaemin, carbachol, A23187, secretin, or VIP (Table III). Furthermore, the action of BtzcGMP appeared to be fully reversible since preincubating acini with BtzcGMP or BtzcGMP plus CCK-OP and then washing the tissue did not alter basal or CCK-OP-stimulated amylase release or the ability of the nucleotide to inhibit the action of CCK-OP determined in a subsequent incubation (Table IV).

In pancreatic acinar cells one of the initial effects of cholecystokinin is to cause a significant increase in calcium outflux (1, 3, 6, 9). In the present studies, a significant increase in ⁴⁰Ca outflux could be detected with 0.1 nM CCK-OP and maximal stimulation occurred with 10 nM CCK-OP (Fig. 6, left). Like its effect on CCK-OP-stimulated amylase secretion, BtzcGMP caused a parallel rightward shift in the dose-response curve for the increase in ⁴⁰Ca outflux caused by CCK OP but did not alter the increase in outflux caused by a maximally effective concentration of CCK-OP (Fig. 6, left). With a fixed
concentration of CCK-OP, increasing concentrations of Bt2cGMP caused a progressive decrease in $^{45}$Ca outflux and with increasing concentrations of CCK-OP, higher concentrations of Bt2cGMP were required to produce detectable inhibition of $^{45}$Ca outflux (Fig. 6, right).

To explore the possibility that the effects of Bt2cGMP were actually due to a contaminant, the material obtained from the commercial supplier was subjected to column and to thin layer chromatography. When chromatographed on DEAE-cellulose, the nucleotide eluted as a single peak at the beginning of the salt gradient (Fig. 7). There was a close correlation between the elution profile of Bt2cGMP determined by absorbance at 254 nm and that of material capable of inhibiting the increase in amylase secretion caused by 0.1 nm CCK-OP (Fig. 7, inset). In each experiment, at least 87% of the applied material was eluted from the column judged by its ability to inhibit stimulation of amylase secretion by CCK-OP and by absorbance at 254 nm. Furthermore, samples eluted from the DEAE-column were as effective in inhibiting the action of CCK-OP as were equal concentrations of stock Bt2cGMP (Table V). On thin layer chromatography, two spots were detected. Approximately 90% of the material had an $R_f$ of 0.60 while the remainder had an $R_f$ of 0.44. Chromatography of $O^\prime$-monobutyryl cyclic GMP gave a single spot at $R_f$ 0.44.

**Reversibility of action of Bt2cGMP on CCK-OP-stimulated amylase release**

Pancreatic acini were preincubated with the indicated agents for 30 min at 37°C and washed twice at ambient temperature with at least 100 volumes of fresh incubation solution (containing no Bt2cGMP or CCK-OP) by alternate centrifugation at 900 × g for 1 min and resuspension. The washed acini were resuspended in standard incubation solution containing the agents specified and amylase release was measured during a 30-min incubation at 37°C. Results were means ± 1 S.D. from four separate experiments.

| Preincubation | Amylase release during incubation | % total |
|---------------|----------------------------------|---------|
|               | No additions | CCK-OP (0.3 mM) | Bt2cGMP (0.10 mM) + CCK-OP | % total |
| No additions  | 4.4 ± 0.6    | 36.3 ± 3.9      | 46.6 ± 0.7       |         |
| Bt2cGMP (3 mM)| 4.7 ± 0.8    | 37.2 ± 4.0      | 47.7 ± 0.5       |         |
| Bt2cGMP + CCK-OP | 4.6 ± 0.6 | 35.9 ± 3.6 | 49.8 ± 0.8 |         |

**Fig. 6.** Effect of Bt2cGMP on the increase in calcium outflux caused by CCK-OP. Acini were suspended in standard incubation solution containing 0.5 mM $^{45}$Ca and were preincubated for 60 min at 37°C. Acini were then washed twice with and resuspended in standard incubation solution. Outflux of $^{45}$Ca was measured during a 5-min incubation at 37°C with the indicated agents. In the left panel the concentrations of Bt2cGMP are given in parentheses. In the right panel the concentrations of CCK-OP are given in parentheses. Results shown are means of quadruplicate determinations from one experiment and this experiment is representative of four others.

**DISCUSSION**

The effects of exogenous cyclic nucleotides on pancreatic enzyme secretion have not been uniform (for review see Ref. 14). This lack of uniformity may reflect, in part, the species of

**TABLE IV**

Comparison of stock Bt2cGMP with chromatographically purified Bt2cGMP in terms of its ability to inhibit amylase secretion stimulated by CCK-OP

Amylase release was measured during a 30-min incubation with the indicated agents at 37°C. Results are means ± 1 S.D. from quadruplicate determinations in one experiment and this experiment is representative of two others. TLC, thin layer chromatography using silica. TLC control was prepared by scraping an area of the plate adjacent to the spot having an $R_f$ of 0.60 and processing this material in the same way as the experimental samples. DEAE, individual fractions from column chromatography using DEAE-cellulose. Concentrations of Bt2cGMP were calculated from the absorbance at 254 nm and an extinction coefficient of 13.5. Concentrations of stock Bt2cGMP were prepared to the same concentration as the chromatographically purified samples.

| Additions | Amylase release | % total |
|-----------|-----------------|---------|
| None      | 2.9 ± 0.4       |         |
| CCK-OP (0.1 mM) | 10.9 ± 0.5     |         |
| +TLC control | 10.8 ± 0.6     |         |
| +Stock Bt2cGMP (0.14 mM) | 5.0 ± 0.4    |         |
| +TLC Bt2cGMP (0.14 mM) | 4.1 ± 0.7    |         |
| +Stock Bt2cGMP (0.06 mM) | 7.7 ± 0.4    |         |
| +DEAE-Bt2cGMP (0.06 mM) | 7.8 ± 0.5    |         |
| +Stock Bt2cGMP (0.10 mM) | 6.4 ± 0.6    |         |
| +DEAE-Bt2cGMP (0.10 mM) | 6.3 ± 0.1    |         |

The sample of Bt2cGMP having a $R_f$ of 0.60 caused the same inhibition of the action of CCK-OP as did an equal concentration of stock Bt2cGMP (Table V).
animal from which the pancreas was obtained, since in some species, increases in endogenous cyclic AMP are not accompanied by corresponding increases in enzyme secretion. For example, Robberecht et al. (15) have found that secretin and VIP can increase cyclic AMP in fragments of pancreas from dog, cat, rat, guinea pig, and mouse, but increase enzyme secretion only from guinea pig and rat pancreas. In the present studies we found that amylase secretion from dispersed pancreatic acini was increased significantly by derivatives of cyclic AMP and by 8Br-cGMP but not by native cyclic AMP, native cyclic GMP or Bt2cGMP. With the exception of our failure to detect an increase in amylase secretion with Bt2cGMP, the present studies agree with previous studies using other preparations of guinea pig pancreas (7, 16, 17).

Dispersed single acinar cells prepared by incubating the pancreas with crude collagenase, crude hyaluronidase, and EGTA differed from dispersed acini prepared with purified collagenase without hyaluronidase or EGTA in terms of their responsiveness to secretagogues as well as the pattern of action of Bt2cGMP (7). In acini the magnitude of the increase in amylase secretion caused by various secretagogues or derivatives of cyclic AMP (severalfold) is substantially greater than that observed previously in single acinar cells (less than 1-fold). Furthermore, unlike its action on amylase release from single acinar cells (7), in pancreatic acini Bt2cGMP did not alter basal enzyme release or the increase in enzyme release caused by VIP or secretin, but inhibited the increase in enzyme release caused by CCK-OP. We do not know the basis for these differences; however, dispersed single acinar cells from mouse pancreas do not have the apical complex of microfilaments and microvilli seen in dispersed acini (18) and the loss of these structures in single acinar cells may be related to their altered responsiveness. In addition, in the present study we found that incubating acini for 20 min with EGTA reduced the magnitude of the increase in amylase release caused by VIP or CCK-OP and modified some, but not all, of the actions of Bt2cGMP. These findings indicate that some of the differences between acini and single acinar cells may be attributable to the calcium-free incubation used to prepare single acinar cells.

In acini from guinea pig pancreas, amylase secretion is increased by agents which increase cellular cyclic AMP (VIP and secretin) and by agents which increase calcium outflux and cellular cyclic GMP (cholinergic agents, cholecystokinin, caerulein, bombesin, litorin, physalaemin, eldoisoin, and A23187) (1-9). When a secretagogue which increases cyclic AMP is combined with a secretagogue which increases calcium outflux, there is potentiation of amylase secretion (7, 9). In contrast, the increase in amylase secretion with maximally effective concentrations of two secretagogues each of which has the same mode of action is equal to that caused by the more effective secretagogue alone (7, 9). In the present studies we found that 8Br-cGMP, like the derivatives of cyclic AMP, mimicked the action of agents which increase endogenous cellular cyclic AMP. Potentiation of amylase secretion occurred when 8Br-cGMP or one of the derivatives of cyclic AMP was combined with a secretagogue which increases calcium outflux and cellular cyclic GMP. The increase in amylase secretion caused by 8Br-cGMP or one of the derivatives of cyclic AMP plus a secretagogue which increases cellular cyclic AMP was the same as that with the secretagogue alone. These effects of 8Br-cGMP were not attributable to its being contaminated with 8Br-cAMP. Furthermore, the time course of action of 8Br-cGMP differed significantly from that of 8Br-cAMP in that with the cyclic GMP derivative no increase in amylase secretion occurred until after 30 min of incubation.

In other tissues, exogenous derivatives of cyclic AMP have been found to cause effects similar to those caused by derivatives of cyclic GMP (for review see Ref. 19), however, in most of these studies it could not be determined whether the response was one which could be produced by both endogenous cyclic nucleotides or, as in the present study, by only one whose effect could be reproduced by exogenous derivatives of cyclic AMP and cyclic GMP. The effects of 6Br-cGMP observed in the present studies illustrate the potential for misinterpreting effects caused by exogenous derivatives of cyclic nucleotides. Since a number of secretagogues can increase cyclic GMP in pancreatic acini and since amylase secretion is increased by 8Br-cGMP, one might conclude that the action of secretagogues which increase endogenous cyclic GMP is, in fact, mediated by cyclic GMP. The present results, however, argue against cyclic GMP being a mediator of the action of cholinergic agents or cholecystokinin since in terms of stimulating amylase secretion, 8Br-cGMP appears to exert this effect by mimicking the action of endogenous cyclic AMP. If cyclic GMP were a mediator of the action of secretagogues such as cholecystokinin and if 8Br-cGMP mimicked the action of endogenous cyclic GMP, we should have seen potentiation of amylase secretion with secretin or VIP plus 8Br-cGMP and not with CCK-OP or carbachol plus cyclic GMP. An unanticipated finding was our observation that Bt2cGMP could competitively inhibit the increase in amylase secretion caused by CCK-OP. In particular, Bt2cGMP caused a parallel, rightward shift in the dose-response curve for CCK-OP-stimulated amylase release, the magnitude of this shift was proportional to the concentration of Bt2cGMP and the nucleotide did not alter the maximal increase in amylase secretion caused by CCK-OP. This inhibition was fully reversible and was specific for cholecystokinin and structurally related peptides. Bt2cGMP did not alter the action of secretagogues which have a mode of action similar to that of cholecystokinin and did not alter the action of secretagogues whose effects are mediated by cyclic AMP. Finally, the pattern of the effect of Bt2cGMP on amylase secretion stimulated by CCK-OP was identical with that of atropine on enzyme secretion stimulated by carbachol. This ability of Bt2cGMP to inhibit the action of CCK-OP was not attributable to the presence of a contaminant in the material obtained from the commercial supplier, since the inhibitory activity co-chromatographed with Bt2cGMP in two different systems.

Inhibition of the action of cholecystokinin by butyryl cyclic GMP required the presence of at least one butyryl moiety. Native cyclic GMP, O'-tyrosine methyl ester cyclic GMP, and O'-sucinyl cyclic GMP did not alter the increase in amylase secretion caused by CCK-OP. Furthermore, the inhibitory potency of butyryl cyclic GMP was determined by the position and number of butyryl groups. Bt2cGMP was 20 times more potent than O'-monobutyryl cyclic GMP which was 5 times more potent than N2-monobutyryl cyclic GMP. Since neither native cyclic GMP nor 8Br-cGMP was able to inhibit the action of cholecystokinin, the ability of Bt2cGMP to antagonize the action of cholecystokinin does not reflect a physiologic action of native cyclic GMP but, instead reflects a pharmacologic activity which is peculiar to butyryl derivatives of cyclic GMP.

Previously, we have found that one of the earliest steps in the mechanism of action of cholecystokinin on pancreatic acinar cells is to effect a significant increase in outflux of exchangeable cellular calcium (1, 3, 5, 6). In the present studies we found that the pattern of action of Bt2cGMP on calcium outflux stimulated by CCK-OP was similar to its pattern of action on cholecystokinin-stimulated amylase secretion. Although Bt2cGMP acts in a reversibly competitive fashion to
inhibit one of the earliest steps in the action of cholecystokinin, we do not know whether the nucleotide derivative is functioning as a full or partial competitive antagonist (20). That is, Bt*cGMP might exert its effects by competing with cholecystokinin for occupation of the cholecystokinin receptor (full competition) or BtzcGMP might interact with sites which are functionally distinct from the cholecystokinin receptor and by so doing reduce the affinity of the cholecystokinin receptor for its ligands (partial competition). Obviously these possibilities as well as others will require additional studies, especially those which examine directly the interaction of cholecystokinin with its receptors on pancreatic acinar cells.

Acknowledgments—We thank Mary Ernst for preparing this manuscript.

REFERENCES
1. Gardner, J. D., Conlon, T. P., Klaeveman, H. L., Adams, T. D., and Ondetti, M. A. (1975) J. Clin. Invest. 56, 366-375
2. Robberecht, P., Conlon, T. P., and Gardner, J. D. (1976) J. Biol. Chem. 251, 4535-4539
3. Christophe, J. P., Frandsen, E. K., Conlon, T. P., Krishna, G., and Gardner, J. D. (1976) J. Biol. Chem. 251, 4640-4645
4. Gardner, J. D., Conlon, T. P., and Adams, T. D. (1976) Gastroenterology 70, 29-35
5. Shelby, H. T., Gross, L. P., Lichty, P., and Gardner, J. D. (1976) J. Clin. Invest. 58, 1482-1493
6. Gardner, J. D., and Hahn, W. F. (1977) Biochim. Biophys. Acta 471, 466-476
7. Gardner, J. D., and Jackson, M. J. (1977) J. Physiol. (Lond.) 270, 439-454
8. May, R. J., Conlon, T. P., Erspamer, V., and Gardner, J. D. (1978) Am. J. Physiol. 235, E112-E118
9. Peikin, S. R., Rottman, A. J., Batzi, S., and Gardner, J. D. (1978) Am. J. Physiol. 235, 743-750
10. Ceska, M., Brown, B., and Birath, K. (1969) Clin. Chim. Acta 26, 445-453
11. Ceska, M., Birath, K., and Brown, R. (1969) Clin. Chim. Acta 26, 437-444
12. Harper, J. F., and Brooker, G. (1975) J. Cyclic Nucleotide Res. 1, 907-918
13. Lopatin, R. N., and Gardner, J. D. (1978) Biochim. Biophys. Acta 543, 465-475
14. Casey, R. M. (1978) Biol. Rev. 53, 211-354
15. Robberecht, P., Deschodt-Lanckman, M., Lammens, M., DeNeef, P., and Christophe, J. (1977) Gastroenterol. Clin. Biol. 1, 519-525
16. Haymovits, A., and Scheele, G. A. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 156-160
17. Jameson, J. D., and Palade, G. E. (1971) J. Cell Biol. 48, 509-522
18. Williams, P. A. (1977) Cell Tissue Res. 170, 453-466
19. Goldberg, N. D., O'Dea, R. F., and Haddox, M. K. (1973) Adv. Cyclic Nucleotide Res. 3, 155-224
20. Dixon, M., and Webb, E. C. (1964) Enzymes, 2nd Ed, pp. 315-359, Academic Press, New York
Actions of derivatives of cyclic nucleotides on dispersed acini from guinea pig pancreas. Discovery of a competitive antagonist of the action of cholecystokinin.
S R Peikin, C L Costenbader and J D Gardner

*J. Biol. Chem.* 1979, 254:5321-5327.

Access the most updated version of this article at http://www.jbc.org/content/254/12/5321.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/254/12/5321.citation.full.html#ref-list-1
Additions and Corrections

**Vol. 254 (1979) 5321-5327**

**Actions of derivatives of cyclic nucleotides on dispersed acini from guinea pig pancreas.**

Steven R. Peikin, Cynthia L. Costenbader, and Jerry D. Gardner

**Page 5324, Table III, Line 6**

The concentration of CCK-7 (deSO₃) should be 0.3 μM instead of 0.3 nM.

The entire table is reproduced here.

**TABLE III**

| Secretagogue          | Amylase release |       |       |
|-----------------------|-----------------|-------|-------|
|                       | Alone           | + BTcGMP (2.5 mM) | % total |
| None                  | 4.5             | 4.5   |       |
| CCK-7 (0.3 nM)        | 53.2            | 6.5   |       |
| CCK-8 (0.3 nM)        | 55.2            | 5.3   |       |
| CCK-10 (1 nM)         | 57.0            | 5.2   |       |
| CCK-33 (3 nM)         | 44.4            | 12.2  |       |
| CCK-7 (deSO₃) (0.3 μM)| 53.5            | 7.2   |       |
| CCK-7 (ser-SO₃) (1 μM)| 52.7            | 6.2   |       |
| Caerulein (0.3 nM)    | 53.2            | 6.4   |       |
| Bombesin (10 nM)      | 43.7            | 39.9  |       |
| Physalaemin (10 nM)   | 13.0            | 13.0  |       |
| A23187 (3 μM)         | 15.1            | 14.5  |       |
| Carbachol (10 μM)     | 50.0            | 52.2  |       |
| Secretin (0.2 μM)     | 29.0            | 30.3  |       |
| VIP (10 nM)           | 27.9            | 27.3  |       |

**Vol. 254 (1979) 8620-8627**

**Spectrin-actin interaction. Phosphorylated and dephosphorylated spectrin tetramer cross-link F-actin.**

Stephen L. Brenner and Edward D. Korn

**Page 8622, Fig. 1**

Due to a printer's error, Part A of Fig. 1 was omitted. The correct figure appears below.

---

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.
Additions and Corrections

Vol. 254 (1979) 5321–5327

Actions of derivatives of cyclic nucleotides on dispersed acini from guinea pig pancreas.

Steven R. Peikin, Cynthia L. Costenbader, and Jerry D. Gardner

Page 5324, Table III, Line 6

The concentration of CCK-7 (deSO₃) should be 0.3 µM instead of 0.3 nM.

The entire table is reproduced here.

| Secretagogue       | Amylase release | + BTcGMP (2.5 mM) |
|--------------------|-----------------|-------------------|
|                    | Alone           | % total           |
| None               | 4.5             | 4.5               |
| CCK-7 (0.3 nM)     | 53.2            | 6.5               |
| CCK-8 (0.3 nM)     | 55.2            | 5.3               |
| CCK-10 (1 nM)      | 57.0            | 5.2               |
| CCK-33 (3 nM)      | 44.4            | 12.2              |
| CCK-7 (deSO₃) (0.3 µM) | 53.5        | 7.2               |
| CCK-7 (ser-SO₃) (1 µM) | 52.7        | 6.2               |
| Caerulein (0.3 nM) | 53.2            | 6.4               |
| Bombesin (10 nM)   | 43.7            | 39.9              |
| Physalaemin (10 nM)| 13.6            | 13.9              |
| A23187 (3 µM)      | 15.1            | 14.5              |
| Carbachol (10 µM)  | 50.0            | 52.2              |
| Secretin (0.2 µM)  | 29.0            | 30.3              |
| VIP (10 nM)        | 27.9            | 27.3              |

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.