Proglumide and benzotript: Members of a different class of cholecystokinin receptor antagonists (pancreatic secretagogues/amylase secretion/gastrin/caerulinein)

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ABSTRACT In dispersed acini from guinea pig pancreas, proglumide (N-p-benzamido-N,N-dipropylglutaramic acid) and benzotript (N-p-chlorobenzoyl-L-tryptophan) caused a rightward shift in the dose–response curve for cholecystokinin-stimulated amylase secretion but did not alter the maximal increase in amylase secretion caused by cholecystokinin. At relatively low concentrations, proglumide did not alter the stimulation of enzyme secretion caused by secretagogues whose effects are mediated by adenosine 3′,5′-monophosphate (e.g., vasoactive intestinal peptide or secretin) and did not alter the stimulation of enzyme secretion caused by secretagogues that have a mode of action similar to that of cholecystokinin but act through different receptors (e.g., bombesin, physalaemin, edeoidin, and ionophore A23187). There was a close correlation between the ability of proglumide or benzotript to inhibit binding of 125I-labeled cholecystokinin to its receptors on pancreatic acini and the abilities of these compounds to inhibit the action of cholecystokinin on enzyme secretion and on calcium outflux. These results indicate that proglumide and benzotript are members of a different class of cholecystokinin receptor antagonists.

Proglumide (Fig. 1) is a derivative of glutamic acid that has been used for approximately 10 years in Europe and Japan to treat patients with peptic ulcers (1, 2). The claimed effectiveness of proglumide has been attributed to its ability to reduce secretion of gastric acid (1, 2); however, the mechanism of action of proglumide has not been established. Some studies have concluded that proglumide inhibits only gastrin-stimulated acid secretion and does not have anticholinergic or antihistamine activity (2–5), whereas other studies report that proglumide can inhibit the stimulation of acid secretion caused by gastrin, histamine, carbamoylcholine, and insulin (3, 6–8). Proglumide has been reported to inhibit gastrin-stimulated acid secretion competitively (3) as well as noncompetitively (5, 9) and to inhibit binding of radiolabeled gastrin to a heterogeneous mixture of gastric mucosal cells as well as to a crude preparation of gastric mucosal membranes (10). Proglumide has also been reported to alter the action of caerulein on gallbladder muscle and gastric pyloric muscle, gastric acid secretion, and pancreatic fluid secretion (11).

Because gastrin, cholecystokinin (CCK), and caerulein have a common COOH-terminal pentapeptide amide (12) and exert their effects on a particular target tissue by interacting with the same class of receptors (13–15), and because proglumide has been reported to inhibit the actions of both gastrin and caerulein (3, 5, 11), we considered the possibility that proglumide is a specific antagonist of the interaction of CCK and structurally related peptides with their cell surface receptors. To examine this possibility we tested the abilities of proglumide and benzotript (Fig. 1), a derivative of tryptophan that has actions similar to those of proglumide (2), to alter the actions of CCK as well as other secretagogues on dispersed acini from guinea pig pancreas. In addition, we compared the actions of proglumide and benzotript to those of Bt2cGMP, a compound that we (13, 16) and others (15–19) have found to be a specific CCK receptor antagonist.

MATERIALS AND METHODS

Materials. Male guinea pigs (175–225 g) were obtained from the Small Animals Section, Veterinary Resources Branch, National Institutes of Health. Heps was obtained from Boehringer Mannheim; purified collagenase (type CLSPA) was from Worthington; carbamoylcholine (carbachol), atropine sulfate, 8-bromoamidino-3′,5′-monophosphate (8Br-cAMP), Bt2cGMP, and soybean trypsin inhibitor were from Sigma; 125I-labeled N-succinimidyl-3-(4-hydroxyphenyl)propionate (1500 Ci/mmol; 1 Ci = 3.7 × 1010 becquerels) was from Amersham/Searle; Eagle’s basal medium (fraction V) was from Gibco; essential vitamin mixture (100 times concentrated) was from Microbiological Associates (Bethesda, MD); glutamine and synthetic human gastrin I (2–17) fragment were from Research Plus Laboratories (Denville, NJ); bovine plasma albumin (fraction V) was from Armour Pharmaceutical (Phoenix, AZ); Phadebas amylase test and Sephadex G-50 (superfine) were from Pharmacia; 45Ca (12.5 mCi/mg) was from New England Nuclear; and synthetic bombesin, physalaemin, edeoidin, and vasoactive intestinal peptide (VIP) were from Peninsula Laboratories (Belmont, CA). Ionophore A23187 was a gift from Robert Hamill (Eli Lilly). Natural porcine CCK (greater than 99% pure) and natural porcine secretin were gifts from Viktor Mutt (Gastrointestinal Hormone Research Unit, Karolinska Institute, Stockholm, Sweden). Synthetic COOH-terminal octapeptide of CCK (CCK-8) was a gift from Miguel Ondetti (squibb Institute for Medical Research, Princeton, NJ). Caerulein was a gift from Roberto de Castiglione (Farnametia, Milan, Italy). Proglumide and benzotript were gifts from Rotta Laboratories (Milan, Italy).

Unless stated otherwise, the standard incubation solution contained 24.5 mM HEPES (pH 7.4), 98 mM NaCl, 6 mM KCl, 2.5 mM NaH2PO4, 5 mM sodium pyruvate, 5 mM sodium fumarate, 5 mM sodium glutamate, 2 mM glucose, 8Br-cAMP, 8-bromoamidino-3′,5′-monophosphate.

Abbreviations: CCK, cholecystokinin; CCK-7 and CCK-8, synthetic COOH-terminal hepta- and octapeptides of CCK; VIP, vasoactive intestinal peptide; Bt2cGMP, N2′,O7′-dibutylguanosine 3′,5′-monophosphate; 8Br-cAMP, 8-bromoamidino-3′,5′-monophosphate.

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solution was equilibrated with 100% O₂ and all incubations were performed with 100% O₂ as the gas phase.

Methods. Dispersed acini from guinea pig pancreas were prepared by using the published procedure (20) with three minor modifications: (i) The digestion solution contained collagenase at a concentration of 0.12 mg/ml. (ii) After digestion solution had been injected into the pancreas, the tissue was incubated with 5 ml of digestion solution for four sequential 10-min periods instead of one 15-min period followed by two 10-min periods. (iii) The tissue was washed with standard incubation solution only after the third 10-min digestion period instead of after each digestion period.

Amylase release was measured by using the reported procedure (20, 21). Acini from the pancreas of one animal were suspended in 150 ml of standard incubation solution and samples (1.0 ml) were incubated with the appropriate agents for 30 min at 37°C. Amylase activity was determined by the method of Ceska et al. (22, 23), using the Phadebas reagent. Amylase release was calculated as the percentage of the amylase activity in the acini at the beginning of the incubation that was released into the extracellular medium during the incubation.

Outflux of ⁴⁵Ca was determined by using the described procedure (24). Acini from the pancreas of one animal were suspended in 10 ml of standard incubation solution and, after the acini were loaded with ⁴⁵Ca, they were washed and incubated with the appropriate agents for 5 min at 37°C. Outflux of ⁴⁵Ca was calculated as the percentage of the ⁴⁵Ca in the acini at the beginning of the 5-min incubation that was released into the extracellular medium during the incubation.

Binding of ¹²⁵I-labeled CCK (¹²⁵I-CCK) to pancreatic acini was determined as described (13). ¹²⁵I-CCK was prepared by using the published modifications (13) of the procedure of Bolton and Hunter (25). The specific activities of the various preparations of ¹²⁵I-CCK used in the present studies were 900–1300 Ci/mmol. Acini from the pancreas of one animal were suspended in 10 ml of standard incubation solution and incubated with the appropriate agents plus 50 pM ¹²⁵I-CCK (approximately 70,000 cpm/ml of cell suspension) for 30 min at 37°C. Non-saturable binding of ¹²⁵I-CCK was determined as the amount of radioactivity associated with the acini when the incubation contained 0.1 μM CCK-8 and was always less than 20% of the total binding. In this paper all values for bound ¹²⁵I-CCK represent saturable binding and were calculated as total binding minus non-saturable binding.

RESULTS

In acini incubated with increasing concentrations of CCK-8, amylase secretion increased, became maximal with 0.3 nM CCK-8, and then decreased at peptide concentrations above 0.3 nM (Fig. 2). Bt₂cGMP (Fig. 2 Left) and proglumide (Fig. 2 Right) caused a rightward shift in the dose–response curve for CCK-8-stimulated amylase secretion, and the magnitude of the shift was proportional to the concentration of Bt₂cGMP or proglumide. Results similar to those illustrated in Fig. 2 with Bt₂cGMP or proglumide were also obtained with benzotript (not shown).

When acini were incubated with a fixed concentration of CCK-8, the pattern of action of Bt₂cGMP or proglumide depended on the concentration of CCK-8 (Fig. 3). With concentrations of CCK-8 that were submaximal or maximal for causing amylase secretion, increasing concentrations of Bt₂cGMP or proglumide produced a progressive decrease in amylase secretion, and each antagonist was more potent at the lower concentration of CCK-8 (Fig. 3). With a concentration of CCK-8 that was supramaximal for causing amylase secretion, adding increasing concentrations of Bt₂cGMP or proglumide caused
amylase secretion to increase, become maximal, and, in the case of Bt2cGMP, then decrease (Fig. 3).

Table 1 illustrates that Bt2cGMP and proglumide inhibited the increase in enzyme secretion caused by secretagogues that interact with CCK receptors on pancreatic acini (i.e., CCK-8, caerulein, and gastrin) but did not alter basal enzyme secretion or the increase in enzyme secretion caused by secretagogues that do not interact with CCK receptors (i.e., physalaemin, eledoisin, bombesin, A23187, carbachol, VIP, secretin, or 8-Br-cAMP).

Previously we have shown that there is a close correlation between the abilities of CCK and structurally related peptides to inhibit binding of \(^{125}\)I-CCK to pancreatic acini and their abilities to cause mobilization of cellular calcium and to stimulate enzyme secretion (13). As illustrated in Fig. 4, there was a close correlation between the abilities of Bt2cGMP, proglumide, and benzotript to inhibit binding of 1 nM \(^{125}\)I-CCK and their abilities to inhibit the increase in calcium outflux and amylase secretion caused by 1 nM CCK. In terms of the abilities of these compounds to antagonize these actions of CCK, the potency of proglumide was similar to that of benzotript (half-maximal inhibition at approximately 500 \(\mu\)M; Fig. 4), and each of these antagonists was approximately one-fifth as potent as Bt2cGMP (half-maximal inhibition at approximately 100 \(\mu\)M; Fig. 4).

In pancreatic acini that have been incubated with relatively high concentrations of CCK-8, washed, and then reincubated in fresh incubation solution containing no CCK-8, there is significant residual stimulation of amylase secretion (26). Furthermore, Bt2cGMP can prevent as well as reverse this CCK-8-induced residual stimulation (26, 27). As illustrated in Fig. 5, we found that proglumide and benzotript can also reverse the residual stimulation of enzyme secretion caused by CCK-8. The dose-response curves for the inhibition caused by proglumide

### Table 1. Effect of Bt2cGMP and proglumide on the stimulation of amylase release caused by various pancreatic secretagogues

<table>
<thead>
<tr>
<th>Secretagogue</th>
<th>Alone</th>
<th>Bt2cGMP (1 mM)</th>
<th>Proglumide (10 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4.9 ± 1.6</td>
<td>4.9 ± 1.6</td>
<td>5.0 ± 1.6</td>
</tr>
<tr>
<td>CCK-8 (0.3 nM)</td>
<td>31.9 ± 9.6</td>
<td>4.9 ± 0.8*</td>
<td>5.7 ± 0.8*</td>
</tr>
<tr>
<td>Caerulein (0.1 nM)</td>
<td>32.3 ± 10</td>
<td>4.9 ± 0.3*</td>
<td>5.4 ± 0.9*</td>
</tr>
<tr>
<td>Gastrin I(2–17) (1 (\mu)M)</td>
<td>35.0 ± 3.6</td>
<td>6.2 ± 3.6*</td>
<td>4.9 ± 0.6*</td>
</tr>
<tr>
<td>Physalaemin (10 nM)</td>
<td>12.6 ± 2.6</td>
<td>12.7 ± 0.6</td>
<td>12.8 ± 0.6</td>
</tr>
<tr>
<td>Eledoisin (1 (\mu)M)</td>
<td>12.6 ± 2.6</td>
<td>13.1 ± 1.1</td>
<td>12.2 ± 1.3</td>
</tr>
<tr>
<td>Bombesin (10 nM)</td>
<td>30.4 ± 5.1</td>
<td>32.4 ± 8.5</td>
<td>29.6 ± 6.4</td>
</tr>
<tr>
<td>A23187 (5 (\mu)M)</td>
<td>11.2 ± 2.5</td>
<td>11.5 ± 1.5</td>
<td>12.3 ± 0.3</td>
</tr>
<tr>
<td>Carbachol (10 (\mu)M)</td>
<td>25.0 ± 1.1</td>
<td>25.2 ± 1.2</td>
<td>25.9 ± 2.1</td>
</tr>
<tr>
<td>VIP (10 nM)</td>
<td>23.8 ± 4.6</td>
<td>23.6 ± 1.7</td>
<td>25.3 ± 2.6</td>
</tr>
<tr>
<td>Secretin (0.3 (\mu)M)</td>
<td>24.1 ± 5.7</td>
<td>25.4 ± 3.8</td>
<td>24.3 ± 2.0</td>
</tr>
<tr>
<td>8Br-cAMP (1 (\mu)M)</td>
<td>24.6 ± 3.3</td>
<td>23.0 ± 2.0</td>
<td>23.0 ± 1.4</td>
</tr>
</tbody>
</table>

*Significantly different (\(P < 0.01\)) from the value obtained with the secretagogues alone by Student's paired \(t\) test.

Acini were incubated for 30 min at 37°C with various pancreatic secretagogues alone or in the presence of Bt2cGMP or proglumide. In each experiment each value was determined in duplicate and results given are means ± SD from three separate experiments.

![Fig. 2. Effect of Bt2cGMP (Left) and proglumide (Right) on CCK-8-stimulated amylase release from pancreatic acini. Acini were incubated for 30 min at 37°C with various concentrations of CCK-8 plus the indicated concentrations of Bt2cGMP or proglumide. In each experiment each value was determined in duplicate and the results given are means from eight separate experiments.](image1)

![Fig. 3. Effect of Bt2cGMP (Left) and proglumide (Right) on CCK-8-stimulated amylase release from pancreatic acini. Acini were incubated for 30 min at 37°C with various concentrations of Bt2cGMP or proglumide plus the indicated concentrations of CCK-8. In each experiment each value was determined in duplicate and the results given are means from four separate experiments.](image2)

![Fig. 4. Abilities of Bt2cGMP, proglumide, and benzotript to inhibit binding of \(^{125}\)I-CCK (\(\bullet\)), CCK-stimulated outflux of \(^{46}\)Ca (\(\triangle\)), and CCK-stimulated amylase secretion (\(\Delta\)). All experiments were performed with incubation mixtures that contained 1 nM CCK plus the indicated concentrations of the antagonist. Values are expressed as the percent of the value obtained with no added antagonist. Results for binding of \(^{125}\)I-CCK are means of eight separate experiments, those for outflux of \(^{46}\)Ca are means of five separate experiments, and those for amylase release are means of four separate experiments.](image3)
and benzotript were parallel to the curve for the inhibition caused by Bt2cGMP (Fig. 5).

Because proglumide has been reported to have anticholinergic effects on gastric acid secretion (3), we examined the effect of proglumide on carbachol-stimulated amylase secretion in greater detail. Proglumide, when tested at a relatively high concentration (10 mM), did not alter the increase in amylase secretion caused by concentrations of carbachol up to 10 μM, but it caused a 25–35% decrease in the stimulation caused by carbachol concentrations of 30 μM or greater (Table 1, Fig. 6). Concentrations of proglumide of 3 mM or less did not alter the increase in amylase secretion caused by any concentration of carbachol tested (Fig. 6). Benzotript, at concentrations up to 1 mM, did not alter carbachol-stimulated amylase secretion (not shown).

![Graph showing amylase release in response to various concentrations of proglumide and benzotript](image)

**Fig. 6.** Effect of proglumide on carbachol-stimulated amylase release. Acini were incubated for 30 min at 37°C with different concentrations of carbachol with or without 10 mM proglumide (Left) or with different concentrations of proglumide plus the indicated concentrations of carbachol (Right). Results given are means of at least four separate experiments.

**DISCUSSION**

In general, the structural requirements for occupation of a peptide hormone receptor are quite stringent in that only other peptides will bind to the hormone receptor, and the peptides that do bind are usually fragments of the hormone or analogues with a similar chemical structure. One exception to this general pattern occurs with the opiate receptors (28). These receptors can interact not only with peptides (enkephalins and other opioid peptides) but also with morphine and structurally related alkaloid compounds. A second apparent exception to the general principle that only other peptides will bind to peptide hormone receptors is the finding that butyryl derivatives of cyclic GMP will competitively inhibit the interaction of CCK and structurally related peptides with their receptors on pancreatic acinar cells (13, 16, 17) as well as other tissues (15, 19).

The present findings indicate that proglumide, a derivative of glutaramic acid (Fig. 1), and benzotript, a derivative of tryptophan (Fig. 1), are specific antagonists of the interaction of CCK and structurally related peptides with CCK receptors on pancreatic acinar cells. As has been reported for Bt2cGMP (16), proglumide and benzotript cause a rightward shift in the dose-response curve for CCK-stimulated amylase secretion but do not alter the maximal increase in amylase secretion caused by CCK. This antagonism is selective for CCK and structurally related peptides that in proglumide does not alter the actions of secretagogues whose effects are mediated by cyclic AMP and does not alter the actions of secretagogues that have a mode of action similar to that of CCK but act through different receptors (29). Proglumide can cause a small reduction in carbachol-stimulated amylase secretion; however, this inhibition is noncompetitive, occurs only with relatively high concentrations of carbachol (above 10 μM) and proglumide (above 3 mM), and does not occur with benzotript. Finally, there is a close correlation between the ability of proglumide or benzotript to inhibit binding of 3H-CCK to its receptors on pancreatic acini and their abilities to inhibit the action of CCK on enzyme secretion and on calcium outflux.

Previously, we found that in acini that have been incubated with CCK, washed, and then reincubated without CCK, there is significant residual stimulation of enzyme secretion and that this residual stimulation can be reversed rapidly by adding Bt2cGMP (26, 27). The present results illustrate that, like Bt2cGMP, proglumide and benzotript can reverse CCK-induced residual stimulation of amylase secretion; therefore, proglumide and benzotript have the same spectrum of actions on CCK-induced changes in acinar cell function as do butyryl derivatives of cyclic GMP (13, 16, 26, 27). In terms of their chemical structures, however, proglumide and benzotript bear no obvious resemblance to butyryl cyclic GMP (Fig. 1); therefore, they appear to be members of a different class of CCK-receptor antagonists. Moreover, because proglumide and benzotript are relatively inexpensive and are active after oral administration to humans (1) and animals (4), these compounds may be useful in defining the role of CCK and related peptides in various physiological processes and may be of therapeutic value in clinical conditions in which antagonizing the actions of CCK might be expected to produce beneficial effects.