Exendin-3, a Novel Peptide from *Heloderma horridum* Venom, Interacts with Vasoactive Intestinal Peptide Receptors and a Newly Described Receptor on Dispersed Acini from Guinea Pig Pancreas

**DESCRIPTION OF EXENDIN-3(9-39) AMIDE, A SPECIFIC EXENDIN RECEPTOR ANTAGONIST***

(Received for publication, October 15, 1990)

Jean-Pierre Kaufman‡‡, Latika Singh‡, and John Eng¶

*From the ‡Department of Medicine, Division of Digestive Diseases, State University of New York-Health Science Center Brooklyn, New York 11203-2098 and the §Solomon A. Berson Research Laboratory, Veterans Affairs Medical Center, Bronx, New York 10468*

Exendin-3 increased cellular cAMP levels and amylase release from dispersed acini from guinea pig pancreas. Low concentrations (0.1–3 nm) caused a 12-fold increase in cAMP, whereas higher concentrations (0.3–3 µm) caused an additional 24-fold increase in cAMP. Maximal cAMP with the highest concentration tested was the same as the maximal response with secretin, vasoactive intestinal peptide (VIP), peptide histidine isoleucine, helodermin, or helospectin-I. In terms of amylase release, exendin-3 had the same efficacy but was the least potent of these peptides. Exendin-3-induced increases in amylase release were inhibited by VIP receptor antagonists and the new peptide (>0.1 µm) competed with radiolabeled VIP for binding sites on dispersed acini. Increasing concentrations of an exendin-3 fragment, exendin-3(9–39) amide, did not increase cAMP or amylase release but inhibited the increase in cAMP observed with 0.1–3 nm exendin-3. The fragment did not alter the effects of other peptides that are known to increase acinar cAMP. We conclude that exendin-3 interacts with at least two receptors on guinea pig pancreatic acini; at high concentrations (>100 nm) the peptide interacts with VIP receptors, thereby causing a large increase in cAMP and stimulating amylase release; at lower concentrations (0.1–3 nm) the peptide interacts with a putative exendin receptor, thereby causing a smaller increase in cAMP of undetermined function. Exendin-3(9–39) amide is a specific exendin receptor antagonist.

In 1981, one of us (J. P. R.) discovered that Gila monster (*Heloderma suspectum*) venom stimulated pancreatic enzyme secretion in vitro (1, 2). Subsequently, several peptides sharing the ability to increase cAMP levels and stimulate amylase release from dispersed pancreatic acini have been isolated from Helodermatidae venoms. These structural analogues of the mammalian peptides VIP¹ and secretin include helospectin-I (3), helospectin-II (3), and helodermin (4).

Recently, another peptide that increases cellular cAMP in dispersed pancreatic acini and stimulates amylase release was discovered in *Heloderma horridum* venom (5). This new peptide, designated exendin-3, shares homology with VIP, secretin, helospectin-I and -II, and helodermin (5). Hence, we thought it likely that this new peptide would interact with the same receptors on pancreatic acini that mediate the actions of VIP and secretin. To elucidate the nature of these interactions, we examined the actions of exendin-3 on dispersed acini from guinea pig pancreas and compared these actions with those of VIP, secretin, PHI, and bioactive peptides from Helodermatidae venoms.

**MATERIALS AND METHODS**

Male Hartley guinea pigs (150–200 g) were obtained from CAM Research Lab Animals, Wayne, NJ; HEPES from Boehringer Mannheim; collagenase (type CLSFA) from Worthington; bovine albumin (fraction V), soybean trypsin inhibitor, and theophylline from Sigma; fetal bovine serum, basal medium (Eagle's) amino acids (100 times concentrated) and essential vitamin solution (100 times concentrated) from Gibco; Phadebas amylase test from Pharmacia LKB Biotechnology Inc.; VIP, helodermin, helospectin-I, PHI, secretin, calcitonin gene-related peptide, growth hormone-releasing factor, [Ac-Tyr-D-Phe]GF, 1-29 amide, and [Ac-Cys-D-Phe]Leu³GF from Peninsula, Belmont, CA; [125I]succinyl-cAMP from Du Pont-New England Nuclear; cAMP antisera from Biomedical Technologies, Stoughton, MA; and Na¹[¹I] from Amersham Corp. Exendin-3 was purified from *Heloderma horridum* venom as described previously (5).

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, 11.5 mM glucose, 5 mM sodium fumarate, 5 mM sodium pyruvate, 5 mM sodium glutamate, 0.5 mM CaCl₂, 2 mM glutamine, 1% (w/v) albumin, 0.01% (w/v) soybean trypsin inhibitor, 1% (v/v) amino acid mixture, and 1% (v/v) essential vitamin mixture. The standard incubation solution was equilibrated with 100% O₂, and all incubations were performed with 100% O₂ as the gas phase.

Dispersed acini from guinea pig pancreas were prepared using methods described previously (6) and suspended in standard incubation solution containing 5 mM theophylline. Amylase release was measured as described previously (6), and amylase activity was determined by the method of Ceska using the Phadebas test (7). Amylase release was calculated as the percent of amylase activity in the acini at the beginning of the incubation that was released into the extracellular medium during the incubation.

Cellular cAMP was determined by radioimmunoassay using the procedure described previously (8). The concentration of acini in the incubate was adjusted to maintain CAMP on the linear portion of the standard curve. Results for cyclic nucleotide content were expressed as the value obtained with a particular agent (experimental) divided by the value obtained with acini incubated with no additions (control). 1-VIP (350–400 Ci/mmol) was prepared by iodinating synthetic VIP using a minor modification (9) of the chloramine-T technique.

*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 1734 solely to indicate this fact.

†To whom reprint and correspondence should be addressed: SUNY-Health Science Ctr., Box 1196, 450 Clarkson Ave., Brooklyn, NY 11203-2098. Tel.: 718–270–1112.

The abbreviations used are: VIP, vasoactive intestinal peptide; PHI, peptide histidine isoleucine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GRF, growth hormone-releasing factor.
Binding of $^{125}$I-VIP to dispersed pancreatic acini was measured using a procedure previously described (10). The acinar suspension was incubated with $100 \mu M$ $^{125}$I-VIP for 30 min at 37°C, a temperature at which binding is maximal by 10 min and remains constant for another 30 min (10). Nonsaturable binding of $^{125}$I-VIP was defined as the amount of radioactivity associated with the acini in the presence of 10 $\mu M$ VIP and was less than 25% of total binding in all experiments. Saturable binding is total binding minus nonsaturable binding.

Exendin-3(9-39) amide was prepared by digesting exendin-3 (150 nmol) with 0.2 $\mu g$ endoproteinase Asp-N (Boehringer Mannheim) in 0.1 ml of 0.1 M ammonium bicarbonate at 20°C for 16 h. The mixture was separated into its two component fragments, exendin-3(1-8) and exendin-3(9-39) amide, by reverse phase high pressure liquid chromatography.

RESULTS

Initial experiments showed that exendin-3 increased cellular cAMP and stimulated amylase release from dispersed acini (5). These effects were potentiated by adding theophylline, an inhibitor of cyclic nucleotide phosphodiesterases. We compared the dose-response curves for increases in cAMP and amylase release with exendin-3 to those for the structurally related peptides VIP, helodermin, helospectin-I, PHI, and secretin (Figs. 1 and 2).

Maximal concentrations of each peptide tested caused an approximately 36-fold increase in cAMP (Fig. 1). With 3 $\mu M$ exendin-3, the highest concentration tested, the increase in cAMP was the same as that observed with maximal concentrations of the other peptides. The same general pattern of potency was observed as has been described previously (11). However, in contrast to previous work (11), we found that helodermin was approximately 4-fold more potent than helospectin-I. In terms of the concentration of each peptide that caused a 50% increase in cAMP, the following order of potency was observed: secretin (0.7 nM) > helodermin (5 nM) > helospectin (20 nM) > PHI (200 nM). As described previously (11), the pattern of action of VIP was biphasic. Half-maximal increases in cAMP were observed with 0.5 nM and 0.2 $\mu M$ VIP in the first and second phases, respectively.

The dose-response curve for the increase in cAMP with exendin-3 was also biphasic. In the first phase, the increase in cAMP was half-maximal with 0.5 $\mu M$ and maximal with 3 nM exendin-3. In the second phase, a further increase in cAMP was detectable with 300 nM exendin-3, and cAMP continued to increase with 3 $\mu M$ exendin-3, the highest concentration tested. Thus in terms of the increase in cAMP, exendin-3 had the same potency and efficacy as VIP in the first phase but was at least 5-fold less potent than VIP in the second phase.

Maximal concentrations of each peptide tested caused an approximately 8-fold increase in amylase release from dispersed pancreatic acini (Fig. 2). With 3 $\mu M$ exendin-3, the highest concentration tested, the increase in amylase release was the same as that observed with maximal concentrations of the other peptides. In terms of the concentration of peptide that caused a 50% increase in enzyme release, the following order of potency was observed: VIP (60 pm) > helodermin (0.4 nM) > helospectin-I (1 nM) > PHI (7 nM) > secretin (60 nM) > exendin-3 (500 nM). In contrast to a previous study (11), we found that helodermin was about 2.5 times more potent than helospectin-I.

In pancreatic acini, occupation of either high-affinity VIP-prefering receptors or secretin-prefering receptors results in increased levels of cellular cAMP (11, see Refs. 12 and 13 for review). However, only occupation of high-affinity VIP receptors results in an increase in a pool of cAMP that stimulates amylase release (11). Thus, the first phase of the increase in cAMP with VIP correlates with VIP-induced increases in amylase release, whereas amylase release with secretin occurs only with higher concentrations of the peptide (>3 nM) that interact with high-affinity VIP receptors (11).

Comparison of the dose-response curves for exendin-3-induced increases in cAMP and amylase release indicates that, in contrast to VIP, the second phase of exendin-induced increases in cAMP correlates with stimulation of amylase release. This is shown in Fig. 3, where these data are expressed as a percentage of the maximal response. Exendin-3 concentrations (0.1-3 nM) that cause an almost 40% increase in cAMP levels do not stimulate amylase release. In contrast, the second phase of exendin-3-induced increases in cAMP observed with peptide concentrations >100 nM is associated with an increase in amylase release. As noted above, previous studies (11-13) indicated that peptides in the VIP-secretin family, including other bioactive peptides from Helodermatidae venoms, stimulate amylase release by interaction with high-affinity VIP-prefering receptors. The next set of experiments was designed to test the hypothesis that the increase in cAMP and stimulation of amylase release observed with exendin-3 concentrations greater than 100 nM (Fig. 3) is a
Exendin-3-induced amylase release was observed with the VIP receptors, we determined the ability of the DeDtide to inhibit the highest concentration (3 FM) of the antagonists used.

VIP and secretin, inhibited binding of 125I-VIP to dispersed calculated additive value receptor antagonists inhibited enzyme release was the same for the three peptides and the patterns of inhibition were similar. Approximately 40% inhibition of VIP-, secretin-, and exendin-3 (12.2 nM) caused by interaction with this class of VIP receptors.

Fig. 4 shows the effect of adding increasing concentrations of two VIP receptor antagonists on the increase in amylase release observed with VIP, secretin, or exendin-3. Acini were incubated with VIP (1 nM), secretin (1 μM), or exendin-3 (1 μM) for 30 min at 37°C, alone or in combination with indicated concentrations of [Ac-Tyr1,Pro2]GRF 1-29 amide (solid symbols) or [4-Cl-D-Phe6,Leu11]VIP (open symbols). Results are expressed as percentage of increase in amylase release caused by VIP (10.2 ± 1.2), secretin (10.7 ± 1.0), or exendin-3 (12.2 ± 0.6) alone. In each experiment, each value was determined in duplicate, and results given are means ± S.E. from three separate experiments.

To examine further the ability of exendin-3 to interact with VIP receptors, we determined the ability of the peptide to inhibit binding of 125I-VIP to dispersed pancreatic acini (Fig. 5). Significant inhibition of binding occurred with 300 nM exendin-3 and 50% inhibition occurred with 5 μM exendin-3. These data indicate that exendin-3 inhibits binding of radiolabeled VIP over the same range of concentrations required to stimulate amylase release.

Having concluded that the second phase of exendin-3-induced increases in cAMP (exendin-3 concentrations >100 nM) is caused by interaction with VIP-preferring receptors, we examined the first phase of exendin-3-induced increases in cAMP that is not associated with stimulation of amylase release (0.1-3 nM exendin-3). To provide further evidence that the first phase of the exendin-3 cAMP dose-response curve is not due to interaction of the new peptide with VIP receptors, we examined the effect of combining VIP and exendin-3 (Table I). The increase in cAMP with 1 nM exendin-3 plus 1 nM VIP was not significantly different from the calculated value obtained by adding the results with these agents acting alone. These data indicate that at these concentrations, VIP and exendin-3 interact with different receptors.

To determine whether the first phase of the exendin-3 cAMP dose-response curve is due to occupation of secretin/

---

**TABLE I**

<table>
<thead>
<tr>
<th>Additions</th>
<th>Cellular cAMP (experimental/control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.0</td>
</tr>
<tr>
<td>VIP, 1 nM</td>
<td>10.7 ± 0.4</td>
</tr>
<tr>
<td>Exendin-3, 1 nM</td>
<td>6.7 ± 0.2</td>
</tr>
<tr>
<td>Calculated VIP +  exendin-3</td>
<td>16.3 ± 0.4</td>
</tr>
<tr>
<td>Observed VIP +  exendin-3</td>
<td>15.5 ± 1.7</td>
</tr>
</tbody>
</table>

*Indicates that the value obtained with the combination of VIP plus exendin-3 is significantly greater than values obtained with VIP or exendin-3 alone (p < 0.05) but not significantly different from the calculated additive value (p > 0.5) (Student's t test).
peptide is approximately 25% of that observed with 1 nM secretin. However, as illustrated in Fig. 7, increasing concentrations of the fragment caused a progressive rightward shift of the dose-response curve without changing the maximal response for the first phase of the exendin-3-induced increase in cAMP. The increase in cAMP with 100 nM exendin-3, associated with interaction at the VIP-preferring receptor was not altered by addition of exendin-3(9-39) amide (Fig. 7). The

Having excluded interaction with either VIP-preferring or secretin-preferring receptors as an explanation for the effects of 0.1–3 nM exendin-3, we hypothesized that this increase in cAMP resulted from interaction of the peptide with a previously undescribed receptor on pancreatic acini. In the course of examining the actions of several exendin-3 fragments on dispersed acini, we found one that appears to serve as a specific exendin receptor antagonist. A carboxyl-terminal fragment, exendin-3(9-39) amide, at concentrations up to 10 μM, did not alter cellular cAMP or amylase release (data not shown). However, as illustrated in Fig. 7, increasing concentrations of the fragment caused a progressive rightward shift of the dose-response curve without changing the maximal response for the first phase of the exendin-3-induced increase in cAMP. The increase in cAMP with 100 nM exendin-3, associated with interaction at the VIP-preferring receptor was not altered by addition of exendin-3(9-39) amide (Fig. 7). The

FIG. 6. Effect of increasing concentrations of exendin-3 on the increase in cellular cAMP in dispersed pancreatic acini observed with 1 nM secretin. Dispersed acini were incubated for 30 min at 37 °C with increasing concentrations of exendin-3 alone (circles) or in combination with 1 nM secretin (squares). In each experiment, each value (solid symbols) was determined in duplicate and results given are means ± S.E. from six separate experiments. Hypothetical values (hollow circles) for exendin-3 acting alone were calculated using the equation \( R = R_{\text{max}} [C/(C + K)] \) where \( R \) is the calculated response, \( R_{\text{max}} \) is the observed maximal response to exendin-3, \( C \) is the concentration of exendin-3, and \( K \) is the value of \( c \) at which the observed response is half-maximal. Hypothetical values (hollow squares) for the effect of exendin-3 on the increase in cAMP stimulated by secretin were calculated assuming that exendin-3 interacts with the same receptors as secretin using the equation \( R = \{(R_{C/K}) + (R_{B/K})\}/[C/(C + K) + (B/K) + 1] \) where \( R \) is the calculated response, \( R_{C} \) is maximal stimulation caused by secretin alone, \( R_{B} \) is maximal stimulation caused by exendin-3 alone, \( C \) is the concentration of secretin, \( B \) is the concentration of exendin-3, \( K_{C} \) is the concentration of secretin that causes a half-maximal increase in cAMP and \( K_{B} \) is the concentration of exendin-3 that causes a half-maximal increase in cAMP (first phase) (17). exp(cont), experimental/control.

FIG. 7. Effect of increasing concentrations of exendin-3(9-39) amide on the dose-response curve for exendin-3-induced increases in cAMP. Acini were incubated with indicated concentrations of agents for 30 min at 37 °C. In each experiment, each value was determined in duplicate and results given are means ± S.E. from three separate experiments.

FIG. 8. Lack of effect of increasing concentrations of exendin-3(9-39) amide on the dose-response curves for secretin- and VIP-induced increases in cAMP. Acini were incubated with indicated concentrations of agents for 30 min at 37 °C. In each experiment, each value was determined in duplicate and results given are means from three separate experiments. Standard error bars have been omitted for purposes of visual clarity.

The most likely hypothesis accounting for the findings presented in this study is that exendin-3 interacts with two

**DISCUSSION**

The most likely hypothesis accounting for the findings presented in this study is that exendin-3 interacts with two...
other peptides, such as VIP, whose actions on enzyme release are mediated by activation of the adenylyl cyclase system (11-exendin-3-induced increases in cAMP is approximately 1 log concentrations >100 nM). Although the dose-response curve for concentration to the right of that for increases in amylase causes an increase in cellular cAMP that stimulates amylase in the sense that increases in enzyme release are observed the following observations: 

1) Low concentrations of exendin-3 (0.1-3 nM) increase acinar cAMP without stimulating amylase release or competing with radiolabeled VIP for binding sites on dispersed acini. 

2) Adding an inhibitor of cyclic nucleotide phosphodiesterases, such as theophylline, results in potentiation of amylase release and the increase in cellular cAMP caused by exendin-3 (5). This potentiation is similar to that observed with other pancreatic secretagogues, such as VIP and secretin, whose actions are mediated by activation of the adenylyl cyclase system (12, 13).

3) Adding increasing concentrations of the VIP receptor antagonists [Ac-Tyr1,D-Phe2]GHRF 1-29 amide and [4-Cl-D-Phe2,Leu3]VIP results in progressive inhibition of exendin-3-induced amylase release. The pattern of this inhibition is similar to that observed when the VIP receptor antagonists are added to equally efficacious concentrations of VIP or secretin.

4) Exendin-3 inhibited the binding of 125I-VIP to dispersed pancreatic acini in a dose-dependent fashion. Moreover, the concentrations of the new peptide that competed with binding of radiolabeled VIP were similar to those that induced the second phase of the increase in cellular cAMP and stimulated amylase release.

In addition to the VIP-prefering receptor, exendin-3 interacts with a second receptor on dispersed acini from guinea pig pancreas. Supporting data for this part of our hypothesis include the following observations: 1) Low concentrations of exendin-3 (0.1-3 nM) increase acinar cAMP without stimulating amylase release or competing with radiolabeled VIP for binding sites on dispersed acini. 2) Combination of 1 nM exendin-3 and 1 nM VIP, concentrations that are maximal for the first phase of the respective cAMP dose-response curves, results in an additive increase in cAMP. This provides further evidence that, at these concentrations, exendin-3 does not interact with VIP-prefering receptors. 3) Addition of 0.01-100 nM exendin-3 to 1 nM secretin does not alter secretin-induced increases in cAMP. This indicates that exendin-3, an agent that has one-third the efficacy of secretin at these concentrations, does not interact with secretin-prefering receptors. 4) Addition of increasing concentrations of exendin-3(9-39) amide causes a progressive, parallel rightward shift in the cAMP dose-response curve caused by 0.1-10 nM exendin-3. Exendin-3(9-39) amide alone does not alter basal acinar cAMP or amylase release. 5) Exendin-3(9-39) amide does not alter the actions of other agents, including VIP and secretin, that are known to increase pancreatic acinar cAMP. The current data indicate the presence of a receptor on dispersed acini from guinea pig pancreas that has not been described previously. This receptor, that we are designating the exendin receptor, mediates a 12-fold increase in cellular cAMP when stimulated with nanomolar concentrations of exendin-3. This increase in cAMP does not stimulate an increase in amylase release. The function, if any, of the increase in cAMP mediated by interaction with this putative exendin receptor is currently undetermined.

In addition to characterizing the interaction of exendin-3 with receptors on dispersed acini from guinea pig pancreas, we have described the actions of the exendin-3 fragment, exendin-3(9-39) amide. The data presented here indicate that this fragment is a potent (IC50 = 20 nM) and specific exendin receptor antagonist. This agent may prove useful for detecting other ligands that interact with this newly described receptor.

**REFERENCES**

Actions Of Exendin-3 On Pancreatic Acini


