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Black widow spider α -latrotoxin: a presynaptic neurotoxin that shares structural homology with the glucagon-like peptide-1 family of insulin secretagogic hormones

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Abstract

α -Latrotoxin is a presynaptic neurotoxin isolated from the venom of the black widow spider *Latrodectus tredecimguttatus*. It exerts toxic effects in the vertebrate central nervous system by depolarizing neurons, by increasing $[Ca^{2+}]_i$ and by stimulating uncontrolled exocytosis of neurotransmitters from nerve terminals. The actions of α -latrotoxin are mediated, in part, by a GTP-binding protein-coupled receptor referred to as CIRL or latrophilin. Exendin-4 is also a venom toxin, and it is derived from the salivary gland of the Gila monster *Heloderma suspectum*. It acts as an agonist at the receptor for glucagon-like peptide-1(7-36)-amide (GLP-1), thereby stimulating secretion of insulin from pancreatic β -cells of the islets of Langerhans. Here is reported a surprising structural homology between α -latrotoxin and exendin-4 that is also apparent amongst all members of the GLP-1-like family of secretagogic hormones (GLP-1, glucagon, vasoactive intestinal polypeptide, secretin, pituitary adenylyl cyclase activating polypeptide). On the basis of this homology, we report the synthesis and initial characterization of a chimeric peptide (Black Widow GLP-1) that stimulates Ca^{2+} signaling and insulin secretion in human β -cells and MIN6 insulinoma cells. It is also reported here that the GTP-binding protein-coupled receptors for α -latrotoxin and exendin-4 share highly significant structural similarity in their extracellularly-oriented amino-termini. We propose that molecular mimicry has generated conserved structural motifs in secretagogic toxins and their receptors, thereby explaining the evolution of defense or predatory strategies that are shared in common amongst distantly related species including spiders, lizards, and snakes. Evidently, the toxic effects of α -latrotoxin and exendin-4 are explained by their ability to interact with GTP-binding protein-coupled receptors that normally mediate the actions of endogenous hormones or neuropeptides.

Keywords

Presynaptic neurotoxin; Peptide hormone; Calcium; Exocytosis; G protein-coupled receptor; Stimulus-secretion coupling; Pancreatic β -cell; Insulin secretion

1. Introduction

The recent cloning [25,27] and characterization [8] of a receptor for α -latrotoxin (α -LTx; a toxin isolated from the venom of the black widow spider *Latrodectus tredecimguttatus*) has revealed surprising primary amino acid sequence homology with a family of GTP-binding protein-coupled receptors (GPCRs) that include receptors for glucagon-like peptide-1(7–36)-amide (GLP-1), glucagon, secretin, vasoactive intestinal polypeptide (VIP), pituitary adenylyl cyclase-activating polypeptide (PACAP), parathyroid hormone (PTH), and calcitonin. α -LTx exerts toxic effects in the vertebrate central nervous system by binding to high-affinity receptors located on presynaptic nerve endings. It promotes Ca^{2+} influx and interacts with components of the exocytotic secretory apparatus ($G_{\text{O}\alpha}$ proteins, syntaxin, synaptotagmin) to stimulate uncontrolled release of neurotransmitters [16,22,32,33]. α -LTx also acts in the endocrine system to stimulate exocytosis of epinephrine from adrenal chromaffin cells [4,28] or insulin from pancreatic β -cells located in the islets of Langerhans [26]. These effects of α -LTx are mediated, in part, by its binding to a receptor designated as latrophilin or CIRL (the Ca^{2+} -independent receptor for latrotoxin) [25,27].

Analysis of the cDNA coding for CIRL indicates that it is a GPCR classifiable according to the G protein coupled receptor database [24] as a member of group IV of the 'B' family of GPCRs. These group IV receptors are orphan receptors, the endogenous ligands for which have yet to be identified. CIRL is closely related in structure to the corticotropin releasing factor type-2 receptor (CRF-R2) that is a member of group I of the 'B' family. The similarity of CIRL and CRF-R2 was first noted on the basis of a comparison of the primary amino acid sequences that define the seven predicted transmembrane spanning regions of the receptors. Subsequently, it was recognized that this region of CIRL also shares significant homology with group II and group III receptors of the 'B' family that include receptors for PTH and GLP-1, respectively.

The fact that CIRL bears similarity to the GLP-1-R suggests that GPCRs of the 'B' family might share in common structural properties that allow their interaction with secretagogic toxins such as α -LTx. The GLP-1-R normally recognizes the intestinally-derived insulinotropic hormone GLP-1(7–36)-amide [1,12,15,18,31,35,39]. Stimulation of the GLP-1-R on pancreatic β -cells produces a rise of $[\text{cAMP}]_i$, an increase of $[\text{Ca}^{2+}]_i$, and exocytosis of insulin [19–21]. Notably, the GLP-1-R is also activated by exendin-4, a toxin isolated from the venom of the Gila monster *Heloderma suspectum* [6,11,14,29]. On the basis of this information, we hypothesized that any similarity of structure evident for CIRL and the GLP-1-R would be reflected in a similarity of structure for α -LTx and exendin-4. To test this, we compared the structures of α -LTx and exendin-4 while also seeking to define which domains of the receptors these toxins interact with.

Our comparison of the primary amino acid sequences of α -LTx and exendin-4 has revealed two conserved epitopes that are potential determinants of receptor-binding activity. More intriguing, these two epitopes are also shown to be conserved in hormones (glucagon, GLP-1, VIP) and neuropeptides (PACAP) that are among the most potent secretagogues yet described. Such observations suggest that novel therapeutic agents might be derived by the synthesis of chimeric peptides in which functional epitopes of toxins and hormones are 'mixed and matched' [5,12,18,36]. Furthermore, we report that toxins or hormones that target GPCRs of the 'B' family are likely to do so by interacting with a previously unreported, but highly conserved domain located in the extracellularly-oriented amino-terminus of the receptors. Therefore, it appears that a similarity of structure exists not only for two seemingly unrelated toxins (α -LTx and exendin-4), but also for the receptors that these toxins interact with. Such observations provide evidence for a convergent form of

molecular evolution, whereby natural selection has generated conserved structural motifs that contribute to toxin-receptor interactions.

2. Materials and methods

2.1. Islet cell culture and single-cell physiology

Human islets of Langerhans were obtained from Dr Camillo Ricordi of the Diabetes Research Institute of the University of Miami School of Medicine. The islets were maintained in a humidified incubator (95% air, 5% CO₂) at 37°C in culture media containing RPMI 1640, 11.1 mM glucose, glutamine, 10% fetal bovine serum, 100 U ml⁻¹ penicillin, and 100 µg ml⁻¹ streptomycin. The islets were dispersed into a single cell suspension by digestion with trypsin-EDTA and by trituration through a fire-polished Pasteur pipette. The cell suspension was plated onto glass coverslips coated with concanavalin A which facilitates adherence of the cells. Experiments were performed after a 16-h equilibration period in the culture media. The media was replaced with a physiological salt solution containing (in mM): 138 NaCl, 5.6 KCl, 2.6 CaCl₂, 1.2 MgCl₂, and 20 HEPES (pH adjusted to 7.4 with NaOH). Cells were exposed to this solution containing 1 µM fura 2-AM (Molecular Probes, Eugene, OR) for 15 min at room temperature. Cells were then washed with fresh saline and experiments were performed at 32°C using a Zeiss IM35 inverted microscope outfitted with a temperature-controlled stage and a 100× Nikon UVF objective. Dual wavelength excitation microspectrofluorimetry was performed ratiometrically on single cells as described previously [21] using an IonOptix Corp. digital video imaging system (Milton, MA). Calibration of the signal was performed using fura 2 pentapotassium salt dissolved in calibration buffer solutions from Molecular Probes. Insulin-containing β-cells were positively identified at the end of the experiment by fluorescence microscopy in combination with immunocytochemistry using a specific anti-insulin antiserum. Test solutions were applied to individual cells from micropipettes using a PicoSpritzer II pneumatic pressure ejection system (General Valve Corp., NJ) as described previously [20].

2.2. Radioimmunoassay for secreted insulin

Secreted insulin was measured by radioimmunoassay (RIA) using porcine ¹²⁵I-insulin (New England Nuclear; NEX104) and a porcine insulin-specific antiserum raised in guinea pigs (Sigma Chemicals 1-8510; at a 1/80000 dilution). Each assay contained 0.1 ml sample, 0.5 ml antiserum, and 0.1 ml buffer composed of 0.05 M potassium phosphate, 0.15 M NaCl, 0.1% sodium azide, 0.5% human serum albumin, pH 7.4. The addition of 0.1 ml ¹²⁵I-insulin (6000 dpm) was delayed by 5 h so that the sensitivity of the assay was increased due to non-equilibrium binding conditions. Each assay was equilibrated for 20 h at 4°C. Tracer bound to the antiserum was separated from non-bound tracer by centrifugation after addition of 0.5 ml of a solution containing 0.25% dextran (MW 70000) and 2.5% activated charcoal (100–400 mesh). Activity in the charcoal pellet was determined using a gamma counter. A standard curve was generated over a concentration range of 0.12–15.2 ng immunoreactive insulin per ml. The IC₅₀ for displacement of ¹²⁵I-insulin binding by non-radioactive insulin was 2.4 ng ml⁻¹.

3. Results

The reported structural similarity between CIRL and the GLP-1-R [25,27] prompted us to investigate a possible correspondence between the primary amino acid sequences of α-LTx and exendin-4. Two such regions of homology were detected. Comparison of α-LTx and exendin-4 using the Align algorithm indicated 42% sequence identity and 58% homology (as judged by conserved amino acid substitutions) when comparing α-LTx residues 970–981

with exendin-4 residues 17–28 (Fig. 1A, left; numbering of amino acid residues as described in [10,11]). This region of α -LTx corresponds to the transition from the 16th to 17th ankyrin-like repeat in the toxin [10]. It is also located within the postulated receptor-binding domain [16] where it is bracketed by two cysteine residues that are likely to play an important role in the process of intramolecular folding. This region of α -LTx is also found within α -latroinsectotoxin (Fig. 1A, left; α -LITx), a black widow spider toxin that elicits massive neurotransmitter release at invertebrate (insect) neuromuscular junctions [23]. On the basis of these observations, it is proposed here that the E-I-V-K-Y-F-V-G-T-L-G-N and E-A-V-R-L-F-I-E-W-L-K-N sequences found within α -LTx and exendin-4 might constitute two binding domains that confer high affinity for CIRL and the GLP-1-R, respectively.

It is noteworthy that the primary amino acid sequence of exendin-4 is also preserved within the carboxy-terminus of the α -LTx precursor molecule. Comparison of α -LTx and exendin-4 using the Align algorithm indicated 40% sequence identity and 60% homology when comparing α -LTx residues 1371–1380 with exendin-4 residues 1–10 (Fig. 1A, left). This H-S-D-G-I-L-T-K-K-L sequence of α -LTx includes the penultimate amino acid residue (Leu₁₃₈₀) at the carboxy-terminus of the precursor molecule [22]. It is generally believed that post translational processing of the α -LTx precursor molecule requires endopeptidase-catalyzed hydrolysis that liberates this carboxy-terminus [10,16]. This generates two fragments: a biologically active $\approx$ 1170 amino acid fragment of M_r 131 kDa that includes the amino-terminus, and a $\approx$ 200 amino acid fragment of 22 kDa that includes the carboxy-terminus for which no biological activity was previously ascribed. Topologically, this H-S-D-G-I-L-T-K-K-L epitope of α -LTx can be viewed in relation to the E-I-V-K-Y-F-V-G-T-L-G-N epitope described above by bending the toxin molecule back upon itself (Fig. 1A, right). By doing so, the two post translational products of α -LTx reproduce the structure of exendin-4 in a highly conserved fashion (Fig. 1A, left and right).

The H-S-D-G-I-L-T-K-K-L and E-I-V-K-Y-F-V-G-T-L-G-N epitopes of α -LTx that closely resemble exendin-4 are similarly conserved in the related helodermin and helospectin-1 venom toxins isolated from *Heloderma* (Fig. 1B, top; [30,38]). Most significantly, these same two regions of homology are well conserved in the family of secretagogic hormones and neuropeptides that include GLP-1, glucagon, VIP, and PACAP. Particularly striking is the similarity of the H-S-D-G-I-L-T-K-K-L sequence of α -LTx with that of the amino-termini of these transmitters (Fig. 1B, bottom). Similarly, a close match also exists for the E-I-V-K-Y-F-V-G-T-L-G-N sequence of α -LTx and the carboxy-termini of these peptide transmitters (Fig. 1B, bottom). In this context, it is noteworthy that the amino- and carboxy-termini are important determinants of biological activity. Truncation of exendin-4 to generate exendin (9–39) converts exendin-4 from an agonist to an antagonist of the GLP-1-R [14,29,35]. In contrast, the ability of GLP-1 to stimulate cAMP production and secretion of insulin is diminished by amino acid substitutions that reduce the peptide's affinity for the GLP-1 receptor (stars, Fig. 1B; [1]).

Black Widow GLP-1 (BW-GLP-1) is a chimeric peptide we synthesized in order to test our hypothesis that 'modular' structural features dictate toxin or peptide hormone bioactivity. The α -LTx residues 970–981 epitope was inserted into the GLP-1 molecule to replace amino acid residues 17–28 of the hormone (Fig. 2). BW-GLP-1 was then tested for its ability to stimulate a rise of $[Ca^{2+}]_i$ in human pancreatic β -cells preincubated in a permissive concentration (7.5 mM) of D-glucose. BW-GLP-1 produced a concentration-dependent rise of $[Ca^{2+}]_i$ ($EC_{50} \approx$ 1 nM) that was reversible and repeatable (Fig. 3A). No such effect was observed when cells were equilibrated at lower concentrations of glucose (2–5 mM). This observation indicates that BW-GLP-1 potentiates the stimulatory effect of glucose metabolism on β -cell Ca^{2+} signaling, as reported previously for wild-type GLP-1 [19–21].

BW-GLP-1 was also tested for its ability to influence the secretion of insulin from MIN6 insulinoma cells, a model system commonly used for analyses of pancreatic β -cell function. BW-GLP-1 was an effective stimulator of insulin secretion (Fig. 3B) provided that the MIN6 cells were equilibrated in a concentration of D-glucose (11.1 mM) that is known to support stimulus-secretion coupling in this cell line. Surprisingly, radioligand binding assays demonstrated that BW-GLP-1 was without effect on the binding of ^{125}I -GLP-1 to the recombinant GLP-1 receptor derived from human cDNA and expressed in transfected HEK-293 cells (data not shown). Therefore, it appears that 'domain swapping' produced a peptide with structural features that allow it to interact with a new type of receptor, the identity of which remains to be determined.

Since the structures of GLP-1 and exendin-4 are found, in part, within α -LTx, we investigated a possible correspondence between the GLP-1-R and CIRL. This analysis was facilitated by a report that CIRL is a N-glycosylated integral membrane protein comprised of an unusual 2-subunit structure [25]. A 7-fold transmembrane spanning domain of M_r 85 kDa is believed to serve as a signal transducing element, whereas an extracellularly-oriented 120-kDa subunit is derived by proteolytic processing of the proreceptor. It was reported that the p85 subunit of CIRL shares 50–60% amino acid sequence homology within the transmembrane spanning regions of various members of the GLP-1-related family of peptide receptors [25,27]. However, α -LTx-binding activity is associated with the p120 subunit [25]. Therefore, it seemed likely that the structural similarity of the GLP-1, exendin-4, and α -LTx peptides might be explained by their interaction with an extracellular domain that is common to both the GLP-1-R and p120 of CIRL.

To test this possibility, a Blast analysis of the Swis-sProt database was conducted using the amino acid sequence of CIRL (1–850) that corresponds to p120 (Blast algorithm provided by the National Center for Biotechnology Information). We noted a previously unreported close correspondence (Fig. 4A) between CIRL sequence 490–510 and an extracellular sequence (51–71) of the corticotropin-releasing factor receptor (CRF-R2). No other sequences of GPCRs outside of this region were detected. A subsequent Blast analysis of the GenBank data base using CIRL 490–510 revealed a highly significant correspondence between CIRL and members of the GLP-1-related family of receptors including GLP-1, VIP, growth hormone-releasing hormone (GHRH), and PTH receptors (Fig. 4A). Remarkably, this region of CIRL is, itself, homologous in structure to cobra (*Naja*) [17,40] and mamba (*Dendroaspis*) [2,34] snake venom toxins (Fig. 4B) that are classified as short neurotoxins [9,40], and which block neuromuscular transmission by interacting with postjunctional cholinergic receptors [2,9,40]. This correspondence is particularly noteworthy in that it includes the canonical G, E, R, C, and P amino acid residues that are characteristic of short neurotoxins isolated from the Elapidae family of snakes (stars, Fig. 4B; [2]).

4. Discussion

We propose that the α -LTx polypeptide can be viewed as possessing two exendin-4-like domains that may be of importance in determining the interaction of α -LTx with its receptor (CIRL). The first domain comprises an E-I-V-K-Y-F-V-G-T-L-G-N sequence and is located within a region of the toxin characterized by multiple ankyrin-like repeats interspersed by clusters of cysteine residues [22]. The second domain comprises an H-S-D-G-I-L-T-K-K-L sequence and is located near the carboxy-terminus of the toxin. Both domains of α -LTx also bear structural similarity to the family of secretagogic hormones that include GLP-1, glucagon, VIP, and PACAP. Exendin-4 is a high affinity agonist for the GLP-1-R [14], and it contains E-A-V-R-L-F-I-E-W-L-K-N and H-G-E-G-T-F-T-S-D-L sequences that are analogous to the corresponding sequences in GLP-1 as well as α -LTx. Therefore, we conclude that α -LTx and exendin-4 are likely to interact with their cognate GPCRs (CIRL,

GLP-1-R) by emulating the two domain structure characteristic of the GLP-1-related family of secretagogic hormones.

It has been proposed that post translational processing of the α -LTx precursor molecule is a necessary prerequisite for generation of toxin with full biological activity [10]. This apparently involves endopeptidase catalyzed hydrolysis that generates two major fragments. The ≈ 1170 amino acid fragment of M_r 131 kDa contains the α -LTx amino-terminus and is thought to be essential for the toxin-mediated formation of ion channels that are permeant to Ca^{2+} . When purified to homogeneity from crude venom, this component is also known to bind to CIRL [25]. The E-I-V-K-Y-F-V-G-T-L-G-N sequence of α -LTx that resembles exendin-4 is found within this 131 kDa fragment at amino acid residues 970–981. This sequence is also located within the domain comprised of amino acid residues 926–1030 that was previously postulated to contain the receptor binding site [16]. It seems reasonable to hypothesize, therefore, that this exendin-like domain of α -LTx is an important determinant of toxin-receptor interactions.

Post translational processing is also proposed to generate an ≈ 200 amino acid fragment of M_r 22 kDa that contains the carboxy-terminus of α -LTx [10]. The biological significance of this fragment is uncertain. Presumably, it is co-secreted with the 131-kDa fragment as a constituent of the venom. The H-S-D-G-I-L-T-K-K-L sequence of α -LTx that resembles exendin-4 includes the penultimate amino acid residue (Leu₁₃₈₀) located at the carboxy-terminus of the 22-kDa fragment. This observation suggests that the 22-kDa fragment of α -LTx might possess a previously unrecognized biological function as a consequence of its ability to interact with CIRL. How this might be achieved is illustrated in Fig. 1A, right. We propose that the crude venom of *Latrodectus* contains the 131 and 22 kDa fragments of the α -LTx precursor. These two fragments align to reproduce the secondary structure of exendin-4, thereby mimicking the process of intramolecular folding that is characteristic of several of the known peptide hormones that bind to GPCRs of the GLP-1-related family. The secondary structures of glucagon, GLP-1, PTH, and calcitonin have been determined, and it is now established that each peptide forms bent 'hairpin' structures so that the amino- and carboxy-termini are in close juxtaposition [3,31]. Such a topology might be reproduced by the concerted action of the two α -LTx fragments interacting with CIRL via the H-S-D-G-I-L-T-K-K-L and E-I-V-K-Y-F-V-G-T-L-G-N sequences. In this manner, the toxin may recapitulate the primary and secondary structures of an as yet to be identified endogenous transmitter that is the authentic ligand for CIRL. Therefore, the structural features of α -LTx that confer binding appear to be based on a modular organizational principal. This conclusion is supported by our demonstration that the α -LTx residues 970–981 'module' can substitute for the corresponding sequence in GLP-1, thereby allowing synthesis of biologically active BW-GLP-1.

BW-GLP-1 exhibited agonist activity when tested for its ability to stimulate a rise of $[\text{Ca}^{2+}]_i$ in human pancreatic β -cells. Similarly, BW-GLP-1 stimulated the secretion of insulin from mouse MIN6 insulinoma cells. Both actions of BW-GLP-1 were dependent on equilibration of cells in saline containing D-glucose, thereby emphasizing the important role metabolism of glucose plays in supporting β -cell stimulus-secretion coupling. Such actions of BW-GLP-1 were unlikely to be mediated by endogenous GLP-1 receptors because BW-GLP-1 failed to displace the binding of ^{125}I -GLP-1 to recombinant GLP-1 receptors expressed in transfected HEK-293 cells. Therefore, BW-GLP-1 may interact with either of three distinct types of receptors: (1) a receptor related in structure to CIRL, (2) a receptor such as the glucagon or gastric inhibitory polypeptide (GIP) receptor, or (3) an orphan receptor. From this standpoint, it is particularly noteworthy that BW-GLP-1, or chimeric peptides similar in design to it, might be useful tools for identification of novel receptors, the peptide ligands of which remain to be discovered.

Our analysis also leads us to propose that three widely divergent evolutionary lines (spiders, lizards, snakes) evolved a common defense/predatory mechanism by which to target GPCRs of the GLP-1-related family. This process involves molecular mimicry on at least one, and possibly two levels. Toxins such as α -LTx and exendin-4 mimic the structure of endogenous ligands, thereby allowing them to target an extracellular domain of GPCRs that might serve as a ligand recognition site. Evidence that this is a ligand recognition site is provided by reports that single amino acid substitutions within this region of the GLP-1 receptor (W72A or W91A; stars in Fig. 4A; [39]) or VIP receptor (W73A or C86G) [7,13] abrogate ligand-binding activity while having no effect on cell surface expression of the receptors. In marked contrast, toxins derived from snake venom mimic this receptor site. This observation prompts speculation that snake toxins act as competitive antagonists of GPCRs in a manner independent of their interaction with postjunctional cholinergic receptors. Although the endogenous ligand of the α -LTx receptor remains undiscovered, it is known that exendin-4 interacts with GLP-1 receptors that regulate insulin secretion [19-21] and appetite [37]. Therefore, it is of considerable interest to assess the potential applicability of peptide hormone/toxin mimetics as therapeutic agents for use in treatment of pathophysiological disorders that include diabetes mellitus and obesity.

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A. Homology of alpha Latrotoxin and Exendin-4

B. A Family of Structurally-Related Toxins and Hormones

Fig. 1.

(A) Primary amino acid sequence homology of α -latrotoxin (α -LTx), exendin-4, and α -latroinsectotoxin (α -LITx). Boxed residues in the alignment are amino acids found in α -LTx either as identical amino acids or as conserved amino acids. On the right is illustrated how the 131-kDa (residues 1–1170) and 22-kDa (residues 1171–1381) fragments of α -latrotoxin may adopt a conformation allowing them to reproduce the primary structure of exendin-4.

(B) On the top is illustrated an alignment demonstrating sequence homology of *Latrodectus* α -latrotoxin and *Heloderma* helodermin and helospectin-1 venom toxins. On the bottom is illustrated an alignment demonstrating sequence homology of α -latrotoxin with members of the glucagon-like peptide-1-related family of secretagogic hormones and neuropeptides. Stars indicate the position of amino acid substitutions that abrogate the biological activity of GLP-1 [1].

Synthesis of Black Widow GLP-1

Fig. 2.

Design of synthetic Black Widow GLP-1 (BW-GLP-1). The α -LTx (970–981) epitope equivalent to EIVKYFVGTLGN was inserted into the GLP-1(7–36)-amide molecule as indicated. It replaces amino acid residues 17–28 equivalent to QAAKEFIAWLVK of the GLP-1(7–36)-amide hormone in which the first residue (H) is designated number 1.

Fig. 3.

(A) Stimulation of Ca^{2+} signaling by BW-GLP-1. Human pancreatic β -cells maintained in primary cell culture were loaded with fura 2 for measurement of $[\text{Ca}^{2+}]_i$. The cells were equilibrated at 32°C in saline containing 7.5 mM D-glucose. BW-GLP-1 (500 pM) was applied for 50 s to individual cells from a micropipette (indicated by the horizontal bars). BW-GLP-1 produced a sustained rise of $[\text{Ca}^{2+}]_i$ upon which large transient rises of $[\text{Ca}^{2+}]_i$ were superimposed. Identical findings were obtained with an equivalent concentration of GLP-1(7–37) ($n = 10$ cells). (B) Stimulation of insulin secretion by BW-GLP-1. MIN6 cells were grown on 12-well Falcon tissue culture plates at $\approx 70\%$ confluence ($0.5\text{--}1.0 \times 10^5$ cells well^{-1}). Secretion of insulin was measured by RIA of the solution bathing the cells. BW-

GLP-1 (10 nM) stimulated the secretion of insulin from cells pre-equilibrated in saline containing 11.1 mM α -glucose, but not 5.5 mM glucose. Values are the averages of triplicate determinations. The experiment was repeated twice and all values were averaged. N.S., not significant. * Significant by the *t*-test, $P < 0.005$.

Fig. 4.

(A) CIRL resembles a conserved motif in the extracellular tail of GLP-1-related GPCRs. CRF-R2, corticotropin-releasing hormone type-2 receptor; GHRH-R, growth hormone-releasing hormone receptor; VIP-R, vasoactive intestinal polypeptide receptor; SEC-R, secretin receptor; DH-R, insect diuretic hormone receptor; PTH-R, parathyroid hormone receptor. (B) Homology of the conserved CIRL motif with venom toxins isolated from cobra or mamba snakes. Stars indicate the canonical G,E,R,C, and P amino acid residues that are characteristic of short neurotoxins isolated from the Elapidae family of snakes. Data base accession numbers are indicated on the far left in A and B.