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Insulinotropic toxins as molecular probes for analysis of glucagon-like peptide-1 receptor-mediated signal transduction in pancreatic β -cells

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Abstract

Cholera toxin, pertussis toxin, mastoparan, maitotoxin, and α -latrotoxin are complex protein or polyether-based toxins of bacterial, insect, or phytoplankton origin that act with high potency at the endocrine pancreas to stimulate secretion of insulin from β -cells located in the islets of Langerhans. The remarkable insulinotropic properties of these toxins have attracted considerable attention by virtue of their use as selective molecular probes for analyses of β -cell stimulus-secretion coupling. Targets of the toxins include heptahelical cell surface receptors, GTP-binding proteins, ion channels, Ca^{2+} stores, and the exocytotic secretory apparatus. Here we review the value of insulinotropic toxins from the perspective of their established use in the study of signal transduction pathways activated by the blood glucose-lowering hormone glucagon-like peptide-1 (GLP-1). Our analysis of one insulinotropic toxin (α -latrotoxin) leads us to conclude that there exists a process of molecular mimicry whereby the 'lock and key' analogy inherent to hormone-receptor interactions is reproduced by a toxin related in structure to GLP-1.

Keywords

cholera toxin; pertussis toxin; mastoparan; maitotoxin; α -latrotoxin; insulin; GLP-1

1. Introduction

Insulinotropic toxins are potent, naturally-occurring substances of animal or plant origin that act at the pancreatic islets of Langerhans to stimulate secretion of the blood glucose-lowering hormone insulin. These toxins target pancreatic β -cells, a subset of endocrine islet cells that act as blood glucose sensors, secreting insulin when exposed to an increase of blood glucose concentration. Insulin is stored in large dense core granules, and insulin exocytosis is triggered by fusion of secretory granules with the plasma membrane, an event initiated by β -cell glucose metabolism. Various hormonal and neural stimuli also trigger secretion of insulin, usually in a manner synergistic with β -cell glucose metabolism. Such stimuli include glucagon-like peptide-1 (GLP-1; an intestinally-derived hormone), glucagon (acting as an intra-islet paracrine factor), and pituitary adenylyl cyclase activating polypeptide (PACAP; a neuropeptide). GLP-1, glucagon, and PACAP exert their insulinotropic actions by interacting with GTP-binding protein-coupled receptors (GPCRs) [1]. The purpose of this review is to

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illustrate how insulinotropic toxins serve as molecular probes for the analysis of glucose-dependent and hormonally-regulated stimulus-secretion coupling in β -cells. A brief synopsis of GLP-1 receptor-mediated signal transduction is offered, thereby providing a framework in which to evaluate toxin action at each step in the GLP-1 signaling cascade. Emphasized are interactions of toxins with GPCRs, GTP-binding proteins (G proteins), second messenger-generating enzymes, and ion channels. Our analysis of one insulinotropic toxin (α -latrotoxin) leads us to conclude that there exists a process of molecular mimicry whereby the 'lock and key' analogy inherent to hormone-receptor interactions is reproduced by a toxin related in structure to GLP-1.

2. Overview of insulinotropic toxins

Insulinotropic toxins include the oligomeric ('A-B') bacterial exotoxins of *Vibrio cholera* (cholera toxin) and *Bordetella pertussis* (pertussis toxin, PTX, also known as islet activating protein). These toxins act enzymatically as ADP-ribosyltransferases to regulate G protein function. An additional insulinotropic toxin isolated from the venom of the wasp *Vespa* is mastoparan. It is a tetradecapeptide that stimulates insulin secretion via effects on G proteins, ion channels, and intracellular Ca^{2+} homeostasis. An insulinotropic toxin of marine origin is maitotoxin, a polyether isolated from the dinoflagellate *Gambierdiscus toxicus*. It is a stimulator of phospholipase C, and it mobilizes intracellular Ca^{2+} stores while simultaneously promoting Ca^{2+} influx. α -Latrotoxin is a 131 kDa protein derived from the venom of the black widow spider *Latrodectus tredecimguttatus*. α -Latrotoxin binds receptors related in structure to GPCRs, intercalates into the plasma membrane to form non-selective cation channels, and stimulates insulin exocytosis. A common denominator underlying the secretagogic action of each of these toxins is their ability to reproduce many of the insulinotropic actions of GLP-1 in β -cells.

3. GLP-1 and β -cell stimulus-secretion coupling

To understand how insulinotropic toxins exert their secretagogic actions in β -cells, it is useful to summarize the signal transduction properties of GLP-1. Two active forms of GLP-1, namely GLP-1-(7–37) and GLP-1-(7–36)-amide, are synthesized by endocrine L-cells of the distal intestine and are secreted into the circulation in response to a meal [2]. GLP-1 acts at the pancreas as a stimulator of insulin secretion, an effect mediated by high affinity receptors for which a single isoform has been identified [3]. The GLP-1 receptor is a 62 kDa GPCR related in structure to the secretin, vasoactive intestinal polypeptide (VIP), and PACAP receptors. GLP-1 receptors also share significant structural homology with orphan GPCRs that bind α -latrotoxin (see below). Peptide hormones isolated from the Gila monster *Heloderma suspectum* are structurally-related to GLP-1 and interact with GLP-1 receptors in a high affinity manner. These peptides include the GLP-1 receptor agonist exendin-4 and the antagonist exendin-(9–39) [4].

Although it is generally accepted that GLP-1 receptors interact with heterotrimeric G proteins, this has not been studied extensively in β -cells. What is known is that β -cells express the G_s and G_{olf} heterotrimers that mediate stimulatory actions of GPCRs on adenylyl cyclase [5–7], and which are substrates for ADP-ribosylation catalyzed by cholera toxin (see below). There is also limited evidence for interactions of GLP-1 receptors with G_i (an inhibitor of adenylyl cyclase that is a substrate for pertussis toxin) and G_q (a stimulator of phospholipase C). As would be expected, occupancy of GLP-1 receptors stimulates adenylyl cyclase, production of cAMP, and activation of protein kinase A (PKA) [8]. Such effects of GLP-1 are reproduced by cholera toxin and pertussis toxin, although the detailed molecular mechanisms of toxin action differ (see below). GLP-1 also stimulates Ca^{2+} influx, mobilization of Ca^{2+} stores, and an increase of $[\text{Ca}^{2+}]_i$ [9,10]. These effects are mimicked by mastoparan and maitotoxin. More

recently, it has become appreciated that GLP-1 stimulates mitogen-activated protein kinases (MAP kinases) and phosphatidylinositol 3-kinase (PI3-kinase) [11,12]. Such actions confer growth factor-like properties to GLP-1, novel effects likely to be of importance in the hormonal induction of stem cell differentiation and β -cell neogenesis. It should be noted that the insulinotropic and growth factor-like properties of GLP-1 have prompted interest in its possible use for treatment of diabetes mellitus. One outgrowth of this line of investigation is the development of peptide chimeras in which receptor-binding domains of insulinotropic hormones are fused to putative effector domains of insulinotropic toxins in an attempt to generate novel therapeutic compounds [13–15].

4. Cholera toxin

Cholera toxin (ChTx) is the prototypic ‘A-B’ insulinotropic toxin for which a molecular mechanism of action has been established [16,17]. ChTx is an oligomer in which a ‘B’ protomer mediates binding of the toxin to ganglioside G_{M1} receptors on the cell surface. Internalization of the holotoxin is accompanied by activation of the ‘A’ protomer, a process involving thiol reduction and subunit dissociation. ChTx acts intracellularly as an NAD: arginine mono-ADP-ribosyltransferase with intrinsic NAD glycohydrolase activity. There is evidence that the enzymatic activity of ChTx reproduces the action of an endogenous mono-ADP-ribosyltransferase, although this remains to be established definitively. ChTx activates a distinct subset of heterotrimeric G proteins, those that mediate stimulatory influences of GPCRs on adenylyl cyclase. These G proteins include G_s and G_{olf} , both of which have been positively identified in rodent β -cells using antisera selective for G protein α subunits. ChTx-catalyzed ADP-ribosylation occurs on Arg residue 201 of G_{sa} , and this modification may lead to decreased affinity of the α -subunit for GDP in addition to inhibiting its intrinsic GTPase activity [18]. The net effect of ChTx-catalyzed ADP-ribosylation is G protein activation with concomitant dissociation of the heterotrimer into its α and $\beta\gamma$ subunits.

It is noteworthy that the predominant ChTx substrates in β -cells are the 45 kDa and 52 kDa isoforms of G_{sa} [7], as well as the 45 kDa G_{olfa} [19]. This molecular heterogeneity is consistent with selective influences of GPCRs on distinct subsets of stimulatory G proteins. Given that β -cells express multiple isoforms of adenylyl cyclases [20–22], including those whose activity is up or down-regulated by Ca^{2+} , it is apparent that proximal steps of receptor-effector coupling in β -cells exhibit a high degree of complexity. As might be expected, G_{sa} and G_{olfa} are enriched in subcellular fractions of β -cell plasma membrane where their interaction with GPCRs and adenylyl cyclase occurs. Plasma membrane expression of G_{sa} and G_{olfa} underlies direct stimulatory effects of ChTx on β -cell cAMP production. This action of the toxin is of paramount importance because cAMP is a necessary ‘competence’ factor for efficient glucose-dependent insulin secretion. Activation of PKA by cAMP results in facilitation of Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCCs) [23], mobilization of Ca^{2+} stores [10,24], and recruitment of secretory granules to active zones of insulin exocytosis [25]. These actions of cAMP, analyzable using ChTx, underlie the established ability of cAMP-elevating hormones such as GLP-1 to render β -cells fully competent to respond to glucose [26].

It is interesting to note that a plasma membrane-delimited action of ChTx may also explain reported inhibitory effects of the toxin on β -cell ATP-sensitive K^+ channels [27] (K-ATP; a complex of Kir6.2 and SUR1). These inwardly-rectifying K^+ channels link glucose-dependent ATP production to cellular depolarization and opening of VDCCs. By activating G_s , ChTx may reproduce the inhibitory effect of GLP-1 on K-ATP [28], thereby promoting Ca^{2+} influx and insulin exocytosis. It is important to note that G_s is also found in β -cell secretory granules [5]. Such a subcellular localization implies that G_s regulates an as yet to be identified late step in stimulus-secretion coupling. This observation raises the attractive possibility that an

endogenous ADP-ribosyltransferase with enzymatic activity similar to that of ChTx might trigger insulin secretion via stimulatory effects on granule-associated G_s and/or G_{olf} .

5. Pertussis toxin

PTX is analogous to ChTx in that it is also an 'A-B' bacterial exotoxin that catalyzes ADP-ribosylation of G protein α -subunits [29]. However, the enzymatic process, G proteins targeted, and functional consequences of PTX treatment are distinct from those of ChTx. The 'A' protomer of PTX catalyzes mono-ADP-ribosylation of G proteins through formation of a thioglycosidic bond between ADP-ribose (derived from β -NAD⁺) and the sulf-hydryl moiety of a consensus cysteine residue located four amino acids from the α -subunit carboxy-terminus. It is this catalytic action of the 'A' protomer that blocks activation of G proteins by cell surface receptors. PTX targets M_r 39–41 kDa α -subunits of G_i and G_o proteins, thereby uncoupling GPCRs from their effectors [30]. Immunological characterization and cDNA cloning of PTX substrates in rat β -cells and insulinoma cells demonstrated the presence of $G_{i\alpha1}$, $G_{i\alpha2}$, and $G_{i\alpha3}$ (mediators of adenylyl cyclase inhibition) as well as three isoforms of $G_{o\alpha}$ [31–34]. PTX shares a similarity with ChTx in that it stimulates cAMP production. However, this is achieved by its ability to uncouple GPCR-mediated inhibition of adenylyl cyclase, not by a direct stimulation of G_s .

The insulinotropic action of PTX was first noticed in studies of the blood glucose-lowering effect of purified extracts of cultured *Bordetella* supernatants [35–37]. Subsequently, it was demonstrated that PTX disinhibits insulin secretion by uncoupling inhibitory influences of β -cell α_2 adrenergic receptors on exocytosis. This insulinotropic effect was positively correlated with disinhibition of adenylyl cyclase and an increase of cAMP production. A possible direct effect of PTX on β -cell glucose metabolism was also suggested by its ability to uncouple α_2 adrenergic inhibition of islet glucose utilization [38]. More recently, it has become appreciated that the secretagogic actions of PTX also include disinhibition of Ca^{2+} influx through VDCCs [39,40]. This effect complements the action of PTX to disrupt stimulatory influences of GPCRs on metabolite-regulated and G protein-coupled inwardly rectifying K^+ channels (K-ATP, GIRK) [41]. It should be noted that PTX also exerts insulinotropic effects at a late step in β -cell stimulus-secretion coupling. Of particular interest are reports demonstrating an ability of PTX to abrogate somatostatin and catecholamine receptor-mediated inhibition of insulin exocytosis in permeabilized β -cells [42,43]. Such findings offer strong support for the role of secretory granule-associated G_i and G_o as direct regulators of exocytosis [44].

6. Mastoparan

Mastoparan is a 14-residue cationic, amphiphilic tetradecapeptide (INLKALAALAKKIL) originally discovered in wasp venom as the agent that induces histamine release from mast cells (reviewed in [45]). Mastoparan stimulates pancreatic insulin secretion, an effect blocked by treatment of islets with PTX [46]. Since PTX is relatively specific for G_i and G_o , this observation raises the surprising possibility that mastoparan acts via G proteins of this subtype. This conclusion is strengthened by a report that PTX blocks insulin secretion stimulated by mastoparan in permeabilized insulinoma cells. Such findings raise an obvious discrepancy. How is it that G_i and G_o subserve stimulatory influences of mastoparan on insulin secretion, while simultaneously mediating inhibitory effects of somatostatin on exocytosis (see above)?

Insight into how mastoparan acts at the molecular level does not fully resolve this discrepancy. Mastoparan activates G_i and G_o by mimicking the intracellular cationic amphiphilic G protein binding domains of GPCRs [47]. These binding domains occur within the second and third intracellular loops of the receptors and also at the carboxy-terminal tail. Mastoparan accelerates G protein α -subunit guanyl nucleotide exchange by facilitating dissociation of GDP, a mechanism of activation analogous to that which occurs when G proteins interact with ligand-

occupied GPCRs. This effect of mastoparan is reported to be blocked by PTX [48]. The transduction process whereby G protein activation occurs is mediated by the α -subunit carboxy terminus, the same region targeted by PTX for ADP-ribosylation. These findings are most simply interpreted in terms of an ability of mastoparan to reproduce the activation of G_i and G_o that occurs following stimulation of cell surface receptors. Receptors that couple to G_i and G_o include those that are specific for somatostatin, galanin, neuropeptide Y, and norepinephrine. However, all of these receptors are known to mediate inhibition of insulin secretion, not stimulation, as is observed with mastoparan. It should be noted that in a lipid environment, mastoparan adopts an amphiphilic α -helical structure critical for full biological activity [49]. This structure may allow interaction of the toxin with other types of G proteins, and might also confer G protein-independent mechanisms of action. Indeed, more recent studies demonstrated PTX-insensitive stimulation of insulin secretion from rat and human pancreatic islets treated with mastoparan [50]. Furthermore, mastoparan has been reported to inhibit Ca^{2+} -ATPase [48] and to inhibit K-ATP channels in a G protein-independent manner [51]. This inhibition of K-ATP channels may result from mastoparan-induced hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂) [52,53], an effect that would increase the sensitivity of K-ATP channels to closure by intracellular ATP [54,55] leading to depolarization and Ca^{2+} influx through L-type Ca^{2+} channels. Mastoparan also induces Ca^{2+} influx that is independent of L-type Ca^{2+} channels [56] and may result from activation of Ca^{2+} -permeant non-selective cation channels [57] that are suggested to play an important role in depolarizing β -cells [58]. However, mastoparan-induced insulin secretion is maintained in the absence of $[Ca^{2+}]_i$ elevation suggesting a direct stimulation of exocytosis by the toxin [56,59]. Therefore, interpretation of the insulinotropic action of mastoparan must be approached with caution in lieu of more precise information concerning its mechanism of action at the site of insulin exocytosis.

7. Maitotoxin

Maitotoxin (MTX) is a polyether toxin that exists in three molecular forms [60,61], and which acts at an extracellular binding site [58,62] via receptors that have yet to be identified [63]. MTX is a powerful stimulator of β -cell Ca^{2+} signaling and insulin secretion. The increase of $[Ca^{2+}]_i$ results from depolarization-induced entry of Ca^{2+} via VDCCs. MTX also triggers VDCC-independent Ca^{2+} influx by activating Na^+ and Ca^{2+} -permeant nonselective cation channels (NSCCs). These effects of MTX are analogous to previously reported actions of GLP-1. It has also been demonstrated that MTX stimulates phosphoinositide hydrolysis and mobilization of inositol trisphosphate (IP₃)-sensitive Ca^{2+} stores [64–66]. More poorly understood effects of the MTX include its ability to stimulate calpain [67] and to activate extracellular signal-regulated protein kinases [68].

Of interest is the possibility that MTX might serve as a pharmacological probe for analysis of signaling events that trigger capacitative Ca^{2+} influx. Depletion of Ca^{2+} stores induces influx of Ca^{2+} across the plasma membrane, and replenishment of Ca^{2+} stores, a process referred to as capacitative Ca^{2+} influx. The MTX-induced increase of $[Ca^{2+}]_i$ is associated with an inward membrane current, as observed in cells voltage clamped at membrane potentials that prevent activation of VDCCs [58,69,70]. These effects of MTX are abrogated by removal of extracellular Na^+ and are diminished by removal of Ca^{2+} , thereby implying that the NSCCs activated by MTX allow permeation by Na^+ and Ca^{2+} , simultaneously. Analysis of single channel currents activated by MTX demonstrated that these NSCCs exhibit a conductance of 25–30 pS [58]. On the basis of these observations, it has been speculated that the NSCCs activated by MTX mediate capacitative Ca^{2+} influx [70]. However, capacitative Ca^{2+} influx is blocked in the RINm5f insulinoma cell line by staurosporine [71], a protein kinase C (PKC) antagonist. In contrast, the action of MTX is not [69]. A possible explanation for this discrepancy is that MTX-induced Ca^{2+} influx is mediated by a staurosporine-insensitive

isoform of PKC such as PKC β , that plays a role in regulating neuronal non-selective cation channels [72].

8. α -Latrotoxin and latrophilin (CIRL)

α -Latrotoxin exerts GLP-1-mimetic actions, effects that include cellular depolarization, activation of NSCCs, an increase of $[Ca^{2+}]_i$, and insulin exocytosis [13,73–75]. A receptor for the toxin, detected in β -cells, and designated as latrophilin or CIRL, bears structural similarity to the GLP-1 receptor [76–78]. Analysis of the CIRL cDNA indicates that the CIRL protein is classifiable as a member of group IV of the ‘B’ subgroup of GPCRs [77]. Group IV receptors are orphan receptors, the endogenous ligands for which have yet to be identified. CIRL is related in structure to the corticotropin releasing factor type-2 receptor (CRF-R2), 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 amino acid sequences that define the seven transmembrane spanning regions of the receptors [77]. Subsequently, it was recognized that this region of CIRL also shares homology with group II and group III receptors of the ‘B’ family, including receptors for parathyroid hormone (PTH) and GLP-1, respectively. A noteworthy feature of ‘B’ type receptors is their established role in secretory processes associated with simultaneous elevations of cAMP and Ca^{2+} concentration.

CIRL also bears resemblance to ‘B’ type GPCRs within its extracellularly-oriented amino terminus that contains the binding site for α -LTX and which is designated as the p120 subunit to differentiate it from the p85 subunit that contains the seven transmembrane spanning domains [13]. A Blast analysis of the p120 subunit of CIRL demonstrated a close correspondence between CIRL amino acid residues 490–510 and an extracellular sequence (51–71) of CRF-R2 (figure 1). No other regions of structural homology were detected. This same analysis also revealed structural homology of CIRL with additional members of ‘B’ type GPCRs, including receptors for GLP-1, secretin, VIP, growth hormone-releasing hormone (GHRH), and PACAP (figure 2). Given that CIRL is an orphan receptor, it is reasonable to speculate that CIRL is targeted by an endogenous ligand, possibly a peptide related in structure to GLP-1. An outgrowth of this speculation is that α -latrotoxin reproduces the biological actions of the endogenous ligand, an effect mediated by the putative CIRL 490–510 ligand recognition site. Evidence that this region of type ‘B’ receptors constitutes a ligand binding domain is provided by reports that single amino acid substitutions within the GLP-1 receptor (W72A or W91A) [79] or VIP receptor (W73A or C86G) [80] abrogate ligand-binding activities while having no effect on cell surface expression of the receptors (figure 2).

Three CIRL-like genes exist and they are designated as CIRL1, CIRL2, or CIRL3 [81–83]. The type-1 isoform is a high-affinity α -latrotoxin receptor, the type-2 isoform is a low-affinity receptor, and the type-3 isoform has little affinity for the toxin. These observations prompted us to examine which isoforms of CIRL are expressed in β -cells. Expression of CIRL mRNA was evaluated by RT-PCR using oligonucleotide primers specific for each receptor subtype. The consistent finding was that expression of CIRL2 mRNA occurs, as detected in the INS-1 insulinoma cell line (figure 3). Evidence was also obtained for expression of CIRL3 in INS-1 cells, whereas CIRL1 was observed in minor quantities (data not shown). It is apparent that the type-2 isoform of CIRL might play a major role as a mediator of α -latrotoxin action in β -cells. Perhaps more intriguing is the role of CIRL3, potentially acting as an orphan receptor for an as yet to be discovered ligand, the properties of which may confer insulinotropic activity.

The fact that CIRL bears structural resemblance to GPCRs of the ‘B’ family prompted us to test for a possible correspondence between α -latrotoxin and hormones or neurotransmitters that interact with receptors of this subtype. In general, peptides that interact with ‘B’ type receptors are viewed as constituting a structurally-related family of GLP-1-like molecules.

These peptides include GLP-1, glucagon, PACAP, and VIP (figure 4). Also included are the *Heloderma* hormones exendin-4, helodermin, and helospectin that act at GLP-1, PACAP, and VIP receptors (figure 4). We observed that the primary amino acid sequence of α -latrotoxin resembles in structure exendin-4, which as noted above, is a GLP-1 receptor agonist. Two regions of exendin-4-like sequence occur within α -latrotoxin. The first sequence corresponds to residues 970–981 homologous to exendin-4 residues 17–28 (figure 5). This region of α -latrotoxin comprises the transition from the 16th to 17th ankyrin-like repeats [84]. It is also located within the postulated receptor-binding domain where it is bracketed by two cysteine residues that are likely to play an important role in the process of intramolecular folding. This same region of α -latrotoxin is also found within α -latroinsectotoxin (figure 5), a *Latrodectus* toxin that elicits transmitter release at insect neuromuscular junctions [84]. On the basis of these observations we propose 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 -latrotoxin and exendin-4 might constitute receptor recognition domains conferring affinity for CIRL and the GLP-1 receptor, respectively.

A second region of exendin-4-like sequence found within α -latrotoxin is localized to the toxin's carboxy-terminus and corresponds to residues 1371–1380 (figure 6). It is homologous to residues 1–10 of exendin-4. This H-S-D-G-I-L-T-K-K-L sequence of α -latrotoxin includes the penultimate amino acid residue (Leu₁₃₈₀) at the carboxy-terminus of the precursor molecule. It is generally believed that posttranslational processing of the α -latrotoxin precursor requires endopeptidase-catalyzed hydrolysis that liberates the carboxy-terminus [84]. This generates two proteins: a biologically active fragment of M_r 131 kDa that includes the amino-terminus, and a smaller fragment of 22 kDa that includes the carboxy-terminus, but for which no biological activity was previously ascribed. Topologically, the H-S-D-G-I-L-T-K-K-L sequence of α -latrotoxin can be viewed in relation to the E-I-V-K-Y-F-V-G-T-L-G-N sequence described above by bending the toxin molecule back upon itself (figures 5, 6). By doing so, the two fragments of α -latrotoxin reproduce the structure of exendin-4 in a conserved fashion.

As noted above, 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 of α -latrotoxin resembling exendin-4 are similarly conserved in the helodermin and helospectin-1 peptides isolated from salivary glands of *Heloderma* (figure 4). Significantly, these same two regions are well-conserved in the family of secretagogic hormones and neuropeptides that include GLP-1, glucagon, PACAP, and VIP (figure 4). Particularly striking is the similarity of the H-S-D-G-I-L-T-K-K-L sequence of α -latrotoxin with that of the amino-terminal sequence of these peptide transmitters. A close match also exists for the E-I-V-K-Y-F-V-G-T-L-G-N sequence of α -latrotoxin and the carboxy-termini of GLP-1, glucagon, VIP, and PACAP. In this context, it is noteworthy that the amino- and carboxy-termini of peptide hormones 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 receptor [4]. In contrast, the ability of GLP-1 to stimulate cAMP production and secretion of insulin is diminished by amino acid substitutions [85] that reduce the peptide's affinity for the GLP-1 receptor (figure 4, indicated by *).

We conclude that α -latrotoxin and exendin-4 are likely to interact with their cognate GPCRs (CIRL, GLP-1 receptor) by emulating the two domain structure characteristic of the GLP-1-related family of secretagogic hormones. The secondary structures of glucagon, GLP-1, PTH, and calcitonin have been determined, and it is established that each peptide forms bent 'hairpin' structures allowing the amino- and carboxy-termini to be in close juxtaposition [86]. Such a topology might be reproduced by the concerted action of the two α -latrotoxin 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 two toxin fragments may recapitulate the primary and secondary structures of an as yet to be identified endogenous transmitter that serves as the authentic ligand for CIRL. A model summarizing this concept is provided in figure 7.

9. Conclusion

As is apparent from the broad content of this review, an extensive literature exists documenting a wide variety of cellular functions altered upon exposure of pancreatic β -cells to insulinotropic toxins. The pleiotropic nature of such toxins is consistent with their targeting of proximal components of GPCR signaling pathways, thereby influencing multiple downstream effectors. Targets of the toxins are likely to include GPCRs, themselves, as is apparently the case for α -latrotoxin. ChTx and PTX influence the next step in plasma membrane-associated signal transduction by virtue of their ability to regulate G protein function. Actions of ChTx and PTX also appear to extend to the insulin-containing secretory granules, although a detailed mechanistic explanation for how ChTx or PTX-sensitive G proteins regulate the process of insulin granule recruitment and exocytosis remains to be provided. The biological actions of mastoparan and maitotoxin are considerably more mysterious, particularly with respect to their direct effects on exocytosis or capacitative Ca^{2+} influx, respectively. A particularly interesting field of research that is an outgrowth of these studies concerns the development of novel therapeutic compounds, the molecular design of which incorporate structural features of insulinotropic toxins. By fusing effector domains of toxins with appropriate targeting domains specific for cell surface or intracellular receptors, it may be possible to exploit the potent secretagogic properties of insulinotropic toxins as novel blood glucose-lowering agents.

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References

- Holz, GG.; Leech, CA. Glucagon-like peptide-1: An insulinotropic hormone with potent growth factor actions at the pancreatic islets of Langerhans. In: Habener, JF.; Miller, CP.; Hussain, MA., editors. Molecular basis of pancreas development and function. Kluwer; Dordrecht: 2000. in press
- Drucker DJ. Glucagon-like peptides. Diabetes 1998;47:159–169. [PubMed: 9519708]
- Thorens B. Expression cloning of the pancreatic β cell receptor for the gluco-incretin hormone glucagon-like peptide-1. Proc Natl Acad Sci USA 1992;89:8641–8645. [PubMed: 1326760]
- Goke R, Fehmann HC, Linn T, Schmidt H, Krause M, Eng J, Goke B. Exendin-4 is a high potency agonist and truncated exendin-(9-39) amide an antagonist at the glucagon-like peptide-1 receptor of insulin-secreting β -cells. J Biol Chem 1993;268:19650–19655. [PubMed: 8396143]
- Astesano A, Regnaud K, Ferrand N, Gingras D, Bendayan M, Rosselin G, Emami S. Cellular and subcellular expression of Golf/Gs and Gq/G11 α -subunits in rat pancreatic endocrine cells. J Histochem Cytochem 1999;47:289–302. [PubMed: 10026232]
- Skoglund G, Basmaciogullari A, Rouot B, Marie JC, Rosselin G. Cell-specific localization of G protein α -subunits in the islets of Langerhans. J Endocrinol 1999;162:31–37. [PubMed: 10396018]
- Walseth TF, Zhang HJ, Olson LK, Schroeder WA, Robertson RP. Increase in Gs and cyclic AMP generation in HIT cells. Evidence that the 45-kDa α -subunit of Gs has greater functional activity than the 52-kDa α -subunit. J Biol Chem 1989;264:21106–21111. [PubMed: 2556393]
- Gromada J, Holst JJ, Rorsman P. Cellular regulation of islet hormone secretion by the incretin hormone glucagon-like peptide-1. Pflugers Arch 1998;435:583–594. [PubMed: 9479010]
- Holz GG, Leech CA, Habener JF. Activation of a cAMP-regulated Ca^{2+} -signalling pathway in pancreatic β cells by the insulinotropic hormone glucagon-like peptide-1. J Biol Chem 1995;270:17749–17757. [PubMed: 7543091]
- Holz GG, Leech CA, Heller RS, Castonguay MA, Habener JF. cAMP-dependent mobilization of intracellular Ca^{2+} stores by activation of ryanodine receptors in pancreatic β -cells. A Ca^{2+} signaling system activated by the insulinotropic hormone glucagon-like peptide-1 (7-37). J Biol Chem 1999;274:14147–14156. [PubMed: 10318832]

11. Buteau J, Roduit R, Susini S, Prentki M. Glucagon-like peptide-1 promotes DNA synthesis, activates phosphatidylinositol 3-kinase and increases transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) DNA binding in beta (INS-1)-cells. *Diabetologia* 1999;42:856–864. [PubMed: 10440129]
12. Frodin M, Sekine N, Roche E, Filloux C, Prentki M, Wollheim CB, Van Obberghen E. Glucose, other secretagogues, and nerve growth factor stimulate mitogen-activated protein kinase in the insulin-secreting β -cell line, INS-1. *J Biol Chem* 1995;270:7882–7889. [PubMed: 7713882]
13. Holz GG, Habener JF. Black widow spider α -latrotoxin: a presynaptic neurotoxin that shares structural homology with the glucagon-like peptide-1 family of insulin secretagogic hormones. *Comp Biochem Physiol* 1998;B121:177–184.
14. Hallbrink M, Saar K, Ostenson CG, Soomets U, Efendic S, Howl J, Wheatley M, Zorko M, Langel U. Effects of vasopressin-mastoparan chimeric peptides on insulin release and G-protein activity. *Regul Pept* 1999;82:45–51. [PubMed: 10458645]
15. Ostenson CG, Zaitsev S, Berggren PO, Efendic S, Langel U, Bartfai T. Galparan: a powerful insulin-releasing chimeric peptide acting at a novel site. *Endocrinology* 1997;138:3308–3313. [PubMed: 9231782]
16. Moss J, Vaughn M. ADP-ribosylation of guanyl nucleotide-binding regulatory proteins by bacterial toxins. *Adv Enzymol* 1988;61:303–379. [PubMed: 3128060]
17. Gill DM, Meren R. ADP-ribosylation of membrane proteins catalyzed by cholera toxin: basis of the activation of adenylate cyclase. *Proc Natl Acad Sci USA* 1978;75:3050–3054. [PubMed: 210449]
18. Bornancin F, Franco M, Bigay J, Chabre M. Functional modification of transducin induced by cholera or pertussis-toxin-catalyzed ADP-ribosylation. *Eur J Biochem* 1992;210:33–44. [PubMed: 1332864]
19. Svoboda M, Garcia-Morales P, Dufrane SP, Sener A, Valverde I, Christophe J, Malaisse WJ. Stimulation by cholera toxin of ADP-ribosylation of membrane proteins, adenylate cyclase and insulin release in pancreatic islets. *Cell Biochem Funct* 1985;3:25–32. [PubMed: 2988811]
20. Leech CA, Castonguay MA, Habener JF. Expression of adenylyl cyclase subtypes in pancreatic β cells. *Biochem Biophys Res Commun* 1999;254:703–706. [PubMed: 9920805]
21. Yang B, He B, Abdel-Halim SM, Tibell A, Brendel MD, Bretzel RG, Efendic S, Hillert J. Molecular cloning of a full length cDNA for human type 3 adenylyl cyclase and its expression in human islets. *Biochem Biophys Res Commun* 1999;254:548–551. [PubMed: 9920776]
22. Emami S, Regnaud K, Ferrand N, Astesano A, Pessah M, Phan H, Boissard C, Garel JM, Rosselin G. Stimulatory transducing systems in pancreatic islet cells. *Ann NY Acad Sci* 1998;865:118–131. [PubMed: 9928004]
23. Suga S, Kanno T, Dobashi Y, Wakui M. GLP-1 (7-36) amide activates L-type Ca^{2+} channels of pancreatic β -cells through cAMP signaling. *Jpn J Physiol* 1997;47:S13–S14. [PubMed: 9266311]
24. Gromada J, Dissing S, Bokvist K, Renstrom E, Frokjaer-Jensen J, Wulff BS, Rorsman P. Glucagon-like peptide-1 increases cytoplasmic calcium in insulin-secreting β TC-3-cells by enhancement of intracellular calcium mobilization. *Diabetes* 1995;44:767–774. [PubMed: 7789644]
25. Hisatomi M, Hidaka H, Niki I. Ca^{2+} /calmodulin and cyclic 3', 5' adenosine monophosphate control movement of secretory granules through protein phosphorylation/dephosphorylation in the pancreatic β -cell. *Endocrinology* 1996;137:4644–4649. [PubMed: 8895328]
26. Holz GG, Leech CA. The insulinotropic hormone glucagon-like peptide-1. Fehmann, HC.; Goke, B., editors. Vol. 13. Karger; Basel: 1997. p. 171-193.
27. Ribalet B, Ciani S. Characterization of the G-protein coupling of a glucagon receptor to the K-ATP channel in insulin secreting cells. *J Membr Biol* 1994;142:395–408. [PubMed: 7707364]
28. Holz GG, Kuhtreiber WM, Habener JF. Pancreatic β -cells are rendered glucose competent by the insulinotropic hormone GLP-1 (7-37). *Nature* 1993;361:362–365. [PubMed: 8381211]
29. Ui M, Katada T, Murayama T, Kurose H, Yajima M, Tamura M, Makamura T, Nogimori K. Islect-activating protein, pertussis toxin: a specific uncoupler of receptor-mediated inhibition of adenylate cyclase. *Adv Cyclic Nucleotide Phosphoryl Res* 1984;17:145–151.
30. Holz GG, Turner TJ. Pertussis toxin-sensitive GTP-binding proteins characterized in synaptosomal fractions of embryonic avian cerebral cortex. *Comp Biochem Physiol* 1998;119B:210–211.

31. Kowluru A, Rabaglia ME, Muse KE, Metz SA. Subcellular localization and kinetic characterization of guanine nucleotide binding proteins in normal rat and human pancreatic islets and transformed beta cells. *Biochim Biophys Acta* 1994;1222:348–359. [PubMed: 8038203]
32. Kowluru A, Seavey SE, Rhodes CJ, Metz SA. A novel regulatory mechanism for trimeric GTP-binding proteins in the membrane and secretory granule fractions of human and rodent β cells. *Biochem J* 1996;313:97–107. [PubMed: 8546716]
33. Berrow NS, Milligan G, Morgan NG. Immunological characterization of the guanine nucleotide binding proteins Gi and Go in rat islets of Langerhans. *J Mol Endocrinol* 1992;8:103–108. [PubMed: 1515016]
34. Terashima T, Katada T, Oinuma M, Inoue Y, Ui M. Endocrine cells in pancreatic islets of Langerhans are immunoreactive to antibody against guanine nucleotide-binding protein (Go) purified from rat brain. *Brain Res* 1987;417:190–194. [PubMed: 3040182]
35. Katada T, Ui M. Effect if in vivo pretreatment of rats with a new protein purified from *Bordetella pertussis* in in vitro secretion of insulin: role of calcium. *Endocrinology* 1979;104:1822–1827. [PubMed: 376293]
36. Katada T, Ui M. Islet-activating protein. Enhanced insulin secretion in pancreatic islets due to activation of native calcium ionophores. *J Biol Chem* 1979;254:469–479. [PubMed: 216676]
37. Katada T, Ui M. Slow interaction of islet-activating protein with pancreatic islets during primary culture to cause reversal of alpha-adrenergic inhibition of insulin secretion. *J Biol Chem* 1980;255:9580–9588. [PubMed: 6253447]
38. Laychock SG, Bilgin S. Alpha 2-adrenergic inhibition of pancreatic islet glucose utilization is mediated by an inhibitory guanine nucleotide regulatory protein. *FEBS Lett* 1987;218:7–10. [PubMed: 2885223]
39. Schmidt A, Hescheler J, Offermanns S, Spicher K, Hinsch KD, Klinz FJ, Codina J, Birnbaumer L, Gausepohl H, Frank R, et al. Involvement of pertussis toxin-sensitive G-proteins in the hormonal inhibition of dihydropyridine-sensitive Ca^{2+} currents in an insulin-secreting cell line (RINm5F). *J Biol Chem* 1991;266:18025–18033. [PubMed: 1680855]
40. Hsu WH, Xiang HD, Rajan AS, Kunze DL, Boyd AE. Somatostatin inhibits insulin secretion by a G-protein-mediated decrease in Ca^{2+} entry through voltage-dependent Ca^{2+} channels in the beta cell. *J Biol Chem* 1991;266:837–843. [PubMed: 1702440]
41. Dunne MJ, Bullett MJ, Li G, Wollheim CB, Petersen OH. Galanin activates nucleotide-dependent K^{+} channels in insulin-secreting cells via a pertussis toxin-sensitive G-protein. *EMBO J* 1989;8:413–420. [PubMed: 2470586]
42. Persaud SJ, Jones PM, Howell SL. Effects of *Bordetella pertussis* toxin on catecholamine inhibition of insulin release from intact and permeabilized rat islets. *Biochem J* 1989;258:669–675. [PubMed: 2543359]
43. Ullrich S, Prentki M, Wollheim CB. Somatostatin inhibition of Ca^{2+} -induced insulin secretion in permeabilized HIT-T15 cells. *Biochem J* 1990;270:273–276. [PubMed: 1975739]
44. Lang J, Nishimoto I, Okamoto T, Regazzi R, Kiraly C, Weller U, Wollheim CB. Direct control of exocytosis by receptor-mediated activation of the heterotrimeric GTPases Gi and Go or by the expression of their active alpha subunits. *EMBO J* 1995;14:3635–3644. [PubMed: 7641683]
45. Ross EM, Higashijima T. Regulation of G-protein activation by mastoparans and other cationic peptides. *Methods Enzymol* 1994;237:26–37. [PubMed: 7935002]
46. Yokokawa N, Komatsu M, Takeda T, Aizawa T, Yamada T. Mastoparan, a wasp venom, stimulates insulin release by pancreatic islets through pertussis toxin sensitive GTP-binding protein. *Biochem Biophys Res Commun* 1989;158:712–716. [PubMed: 2493246]
47. Higashijima T, Uzu S, Nakajima T, Ross EM. Mastoparan, a peptide toxin from wasp venom, mimics receptors by activating GTP-binding regulatory proteins (G proteins). *J Biol Chem* 1988;263:6491–6494. [PubMed: 3129426]
48. Longland CL, Mezna M, Michelangeli F. The mechanism of inhibition of the Ca^{2+} -ATPase by mastoparan. Mastoparan abolishes cooperative Ca^{2+} binding. *J Biol Chem* 1999;274:14799–14805. [PubMed: 10329678]

49. Wakamatsu K, Okada A, Miyazawa T, Ohya M, Higashijima T. Membrane bound conformation of mastoparan-X, a G-protein-activating peptide. *Biochemistry* 1992;31:5654–5660. [PubMed: 1610813]
50. Straub SG, James RF, Dunne MJ, Sharp GW. Glucose augmentation of mastoparan-stimulated insulin secretion in rat and human pancreatic islets. *Diabetes* 1998;47:1053–1057. [PubMed: 9648828]
51. Eddlestone GT, Komatsu M, Shen L, Sharp GW. Mastoparan increases the intracellular free calcium concentration in two insulin-secreting cell lines by inhibition of ATP-sensitive potassium channels. *Mol Pharmacol* 1995;47:787–797. [PubMed: 7723740]
52. Norgauer J, Eberle M, Lemke HD, Aktories K. Activation of human neutrophils by mastoparan. Reorganization of the cytoskeleton, formation of phosphatidylinositol 3,4,5-trisphosphate, secretion up-regulation of complement receptor type 3 and superoxide anion production are stimulated by mastoparan. *Biochem J* 1992;282:393–397. [PubMed: 1312328]
53. Wallace MA, Carter HR. Effects of the wasp venom peptide, mastoparan, on a phosphoinositide-specific phospholipase C purified from rabbit brain membranes. *Biochim Biophys Acta* 1989;1006:311–316. [PubMed: 2557077]
54. Huang CL, Feng S, Hilgemann DW. Direct activation of inward rectifier potassium channels by PIP₂ and its stabilization by Gβγ. *Nature* 1998;391:803–806. [PubMed: 9486652]
55. Baukowitz T, Schulte U, Oliver D, Herlitze S, Krauter T, Tucker SJ, Ruppersberg JP, Fakler B. PIP₂ and PIP as determinants for ATP inhibition of K_{ATP} channels. *Science* 1998;282:1141–1144. [PubMed: 9804555]
56. Komatsu M, McDermott AM, Gillison SL, Sharp GW. Mastoparan stimulates exocytosis at a Ca²⁺-independent late site in stimulus-secretion coupling. Studies with the RINm5F beta-cell line. *J Biol Chem* 1993;268:23297–23306. [PubMed: 8226853]
57. Nie L, Kanzaki M, Shibata H, Kojima I. Activation of calcium-permeable cation channel by insulin in Chinese hamster ovary cells expressing human insulin receptors. *Endocrinology* 1998;139:179–188. [PubMed: 9421413]
58. Leech CA, Habener JF. A role for Ca²⁺-dependent non-selective cation channels in regulating the membrane potential of pancreatic β-cells. *Diabetes* 1998;47:1066–1073. [PubMed: 9648830]
59. Jones PM, Mann FM, Persaud SJ, Wheeler-Jones CP. Mastoparan stimulates insulin secretion from pancreatic beta-cells by effects at a late stage in the secretory pathway. *Mol Cell Endocrinol* 1993;94:97–103. [PubMed: 8397124]
60. Lewis RJ, Holmes MJ, Alewood PF, Jones A. Ionspray mass spectroscopy of ciguatera-1, maitotoxin-2 and -3, and related marine polyether toxins. *Nat Toxins* 1994;2:56–63. [PubMed: 8075894]
61. Holmes MJ, Lewis RJ. Purification and characterization of large and small maitotoxins from cultured *Gambierdiscus toxicus*. *Nat. Toxins* 1994;2:64–72.
62. Murata M, Sasaki M, Yokoyama A, Iwashita T, Gusovsky F, Daly JW, Yasumoto T. Partial structures and binding studies of maitotoxin, the most potent marine toxin. *Bull Soc Pathol Exot* 1992;85:470–473. [PubMed: 1340347]
63. Escobar LI, Salvador C, Martinez M, Vaca L. Maitotoxin, a cationic channel activator. *Neurobiology* 1998;6:59–74. [PubMed: 9713832]
64. Gusovsky F, Yasumoto T, Daly JW. Calcium-dependent effects of maitotoxin on phosphoinositide breakdown and on cyclic AMP accumulation in PC12 and NCB-20 cells. *Mol Pharmacol* 1989;36:44–53. [PubMed: 2546052]
65. Gusovsky F, Yasumoto T, Daly JW. Maitotoxin, a potent, general activator of phosphoinositide breakdown. *FEBS Lett* 1989;243:307–312. [PubMed: 2537233]
66. Sasakawa N, Nakaki T, Kato R. Stimulus-responsive and rapid formation of inositol pentakisphosphate in cultured adrenal chromaffin cells. *J Biol Chem* 1990;265:17700–17705. [PubMed: 2120216]
67. Wang KKW, Nath R, Raser KJ, Hajimohammadreza I. Maitotoxin induces calpain activation in SH-SY5Y neuroblastoma cells and cerebrocortical cultures. *Arch Biochem Biophys* 1996;331:208–214. [PubMed: 8660700]

68. Malaguti C, Yasumoto T, Paolo Rossini G. Transient Ca^{2+} -dependent activation of ERK1 and ERK2 in cytotoxic responses induced by maitotoxin in breast cancer cells. *FEBS Lett* 1999;458:137–140. [PubMed: 10481052]
69. Leech CA, Habener JF. Insulinotropic glucagon-like peptide-1-mediated activation of non-selective cation currents in insulinoma cells is mimicked by maitotoxin. *J Biol Chem* 1997;272:17987–17993. [PubMed: 9218425]
70. Worley JF, Mc Intyre MS, Spencer B, Dukes ID. Depletion of intracellular Ca^{2+} stores activates a maitotoxin-sensitive nonselective cationic current in β -cells. *J Biol Chem* 1994;269:32055–32058. [PubMed: 7798198]
71. Bode HP, Goke B. Protein kinase C activates capacitative calcium entry in the insulin secreting cell line RINm5F. *FEBS Lett* 1994;339:307–311. [PubMed: 8112472]
72. Wang YX, Dhulipala PDK, Li L, Benovic JL, Kotlikoff ML. Coupling of M2 muscarinic receptors to membrane ion channels via phosphoinositide 3-kinase γ and atypical protein kinase C. *J Biol Chem* 1999;274:13859–13864. [PubMed: 10318793]
73. Lang J, Ushkaryov Y, Grasso A, Wollheim CB. Ca^{2+} -independent insulin exocytosis induced by α -latrotoxin requires latrophilin, a G protein-coupled receptor. *EMBO J* 1998;17:648–657. [PubMed: 9450990]
74. Davletov BA, Meunier FA, Ashton AC, Matsushita H, Hirst WD, Leliana VG, Wilkin GP, Dolly JO, Ushkaryov YA. Vesicle exocytosis stimulated by α -latrotoxin is mediated by latrophilin and requires both external and stored Ca^{2+} . *EMBO J* 1998;14:3909–3920. [PubMed: 9670008]
75. Liu J, Mislis S. α -latrotoxin alters spontaneous and depolarization-evoked quantal release from adrenal chromaffin cells: evidence for multiple modes of action. *J Neurosci* 1998;18:6113–6125. [PubMed: 9698306]
76. Davletov BA, Shamotienko OG, Leliana VG, Grishin EV, Ushkaryov YA. Isolation and biochemical characterization of a Ca^{2+} -independent α -latrotoxin-binding protein. *J Biol Chem* 1996;271:23239–23245. [PubMed: 8798521]
77. Krasnoperov VG, Bittner MA, Beavis R, Kuang Y, Salnikow KV, Chepurny OG, Little AR, Plotnikov AN, Wu D, Holz RW, Petrenko AG. α -latrotoxin stimulates exocytosis by the interaction with a neuronal G-protein-coupled receptor. *Neuron* 1997;18:925–937. [PubMed: 9208860]
78. Leliana VG, Davletov BA, Sterling A, Rahman MA, Grishin EV, Totty NF, Ushkaryov YA. α -latrotoxin receptor, latrophilin, is a novel member of the secretin family of G protein-coupled receptors. *J Biol Chem* 1997;272:21504–21508. [PubMed: 9261169]
79. Wilmen A, Van Eyll B, Goke B, Goke R. Five out of six tryptophan residues in the N-terminal extracellular domain of the rat GLP-1 receptor are essential for its ability to bind GLP-1. *Peptides* 1997;18:301–305. [PubMed: 9149304]
80. Couvineau A, Gaudin P, Mauret JJ, Rouyer-Fessard C, Nicole P, Laburth M. Highly conserved aspartate 68, tryptophan 73, and glycine 109 in the N-terminal extracellular domain of the human VIP receptor are essential for its ability to bind VIP. *Biochem Biophys Res Commun* 1995;206:246–252. [PubMed: 7818527]
81. Sugita S, Ichtchenko K, Khvotchev M, Sudhof TC. α -Latrotoxin receptor CIRL/latrophilin 1 (CL1) defines an unusual family of ubiquitous G-protein-linked receptors. G-protein coupling not required for triggering exocytosis. *J Biol Chem* 1998;273:32715–32724. [PubMed: 9830014]
82. Matsushita H, Leliana VG, Ushkaryov YA. The latrophilin family: multiply spliced G protein-coupled receptors with differential tissue distribution. *FEBS Lett* 1999;443:348–352. [PubMed: 10025961]
83. Ichtchenko K, Bittner MA, Krasnoperov V, Little AR, Chepurny O, Holz RW, Petrenko AG. A novel ubiquitously expressed α -latrotoxin receptor is a member of the CIRL family of G-protein-coupled receptors. *J Biol Chem* 1999;274:5491–5498. [PubMed: 10026162]
84. Dulubova IE, Krasnoperov VG, Khvotchev MV, Pluzhnikov KA, Volkova TM, Grishin EV, Vais H, Bell DR, Usherwood PNR. Cloning and structure of δ -latroinsectotoxin, a novel insect-specific member of the latrotoxin family. *J Biol Chem* 1996;271:7535–7543. [PubMed: 8631785]
85. Adelhorst K, Hedegaard BB, Knudsen LB, Kirk O. Structure-activity studies of glucagon-like peptide-1. *J Biol Chem* 1994;269:6275–6278. [PubMed: 8119974]

86. Barden JA, Cuthbertson RM, Jia-Zhen W, Moseley JM, Kemp BE. Solution structure of parathyroid hormone related protein (residues 1-34) containing an Ala substituted for Ile in position 15 (PTHrP [Ala¹⁵]-1-34). J Biol Chem 1996;272:29572–29578. [PubMed: 9368020]

Figure 1.

Correspondence of CIRC1 (RNU72487) with the type 2 CRF receptor (CRF-R2; U16253). The p120 subunit of CIRC1 includes amino acid residues 1–850 in association with the predicted transmembrane-spanning domains (TMSDs). Residues 490–510 of CIRC may constitute a ligand-binding domain (?). A Blast(p) analysis of the GenBank data base demonstrated primary amino acid sequence homology of CIRC1 and CRF-R2, as indicated by identical or conserved amino acid substitutions (vertical bars).

CIRL 490-510	-	W	P	A	T	Q	Q	G	M	L	V	E	R	P	C	P	K	G	T	R	G	I	-
CRF-R2 51-71	-	W	P	Q	S	A	P	G	A	L	V	E	R	P	C	P	E	Y	F	N	G	I	-
GHRH-R 65-85	-	W	P	P	T	G	S	G	Q	W	V	S	L	P	C	P	E	F	F	S	H	F	-
VIP-R1 73-93	-	W	P	A	T	P	R	G	Q	V	V	V	L	A	C	P	L	I	F	K	L	F	-
SEC-R 76-96	-	W	P	S	S	V	P	G	R	M	V	E	V	E	C	P	R	F	L	R	M	L	-
GLP-1-R	-	W	P	D	G	E	P	G	S	F	V	N	V	S	C	P	W	Y	L	P	W	A	-
		*																				*	

Figure 2.

CIRL1 490–510 resembles a conserved motif in the extracellular tail of CRF-R2-related G protein-coupled receptors. Bracketed residues indicate amino acids that are identical to or conserved in comparison to CIRL. Stars (*) indicate amino acid residues within the GLP-1 receptor that abrogate binding of GLP-1 when substituted for by alanine. GHRH (L01407), VIP-R1 (P32241), Sec-R (secretin receptor, U13989), GLP-1-R (P32301).

Figure 3.

RT-PCR analysis of CIRC2 mRNA expression in the INS-1 insulinoma cell line. INS-1 mRNA was extracted using Trizol reagent (Gibco-BRL), and synthesis of cDNA was accomplished using AMV reverse transcriptase and oligo(dT) primers (Promega Reverse Transcriptase System, A3500). PCR amplification of cDNA was initiated using Taq DNA polymerase in combination with deoxyoligonucleotide primers selective for CIRC2 (AF063102). Primer pair 1 corresponds to CIRC2 618–640 (forward) and 1092–1074 (reverse). Primer pair 2 corresponds to CIRC2 111–134 and 690–668. Primer pair 3 corresponds to CIRC2 1576–1596 and 2354–2333. The predicted sizes of the PCR products for primer pairs 1, 2, and 3 are 475, 580, and 779 bp, respectively. PCR products were resolved by agarose gel electrophoresis, gel-purified, and ligated in a sequencing vector (TOPO TA Cloning Kit, Invitrogen Corp.) for sequencing. All three PCR products were confirmed to be identical to CIRC2.

Figure 4.

The GLP-1-like family of structurally-related hormones and neuropeptides. Illustrated are sequence alignments of GLP-1-(7-36)-amide, glucagon, PACAP-27, and VIP. Also illustrated are the lizard hormones exendin-4, helodermin, and helospectin. Bracketed are amino acids identical or conserved within GLP-1. Stars (*) indicate amino acid residues within GLP-1 that are critical for receptor affinity and agonist efficacy.

Figure 5.

Identification of a structural motif within the 131-kDa fragment of α-latrotoxin (α-LTX) that resembles exendin-4 and α-latroinsectotoxin (α-LIT). The linearized α-LTX 131 kDa and 22 kDa fragments derived by proteolytic cleavage are depicted below in a hairpin topology that aligns α-LTX with exendin-4 (not drawn to scale). Illustrated above are amino acid residues 970–981 in α-LTX that are identical to or conserved within exendin-4 residues 17–28 and residues 952–962 of α-LIT (vertical bars).

Figure 6.

Identification of a structural motif within the 22 kDa α -LTX fragment that resembles exendin-4. The linearized α -LTX 131 kDa and minor 22 kDa fragments derived by proteolytic cleavage are again depicted in a hairpin topology that aligns α -LTX with exendin-4. Illustrated are amino acid residues 1371–1380 in α -LTX that are identical to or conserved within exendin-4 residues 1–10 (vertical bars).

Figure 7.

A model depicting the proposed interaction of 'B-type' G protein coupled receptors with ligands that exhibit a two-domain structure analogous to that of GLP-1. The CIRL1 residues 490–510 extracellular domain comprises WPATQQGMLVERPCPKGTRGI. This region of CIRL1 is proposed to interact with an endogenous ligand, the structure of which resembles hormones or neuropeptides of the GLP-1-like family. The endogenous ligand adopts a two-domain hairpin structure that confers receptor binding affinity and which is mimicked in structure by α-LTX residues 1371–1380 and/or 970–981.