Stimulation of neurotransmitter release by α-latrotoxin requires its binding to the calcium-independent receptor of α-latrotoxin (CIRL), an orphan neuronal G protein-coupled receptor. CIRL consists of two noncovalently bound subunits, p85, a heptahelical integral membrane protein, and p120, a large extracellular polypeptide with domains homologous to lectin, olfactomedin, mucin, the secretin receptor family, and a novel structural motif common for large orphan G protein-coupled receptors. The analysis of CIRL deletion mutants indicates that the high affinity α-latrotoxin-binding site is located within residues 467–891, which comprise the first transmembrane segment of p85 and the C-terminal half of p120. The N-terminal lectin, olfactomedin, and mucin domains of p120 are not required for the interaction with α-latrotoxin. Soluble p120 and all its fragments, which include the 467–770 residues, bind α-latrotoxin with low affinity suggesting the importance of membrane-embedded p85 for the stabilization of the complex of the toxin with p120. Two COOH-terminal deletion mutants of CIRL, one with the truncated cytoplasmic domain and the other with only one transmembrane segment left of seven, supported both α-latrotoxin-induced calcium uptake in HEK293 cells and α-latrotoxin-stimulated secretion when expressed in chromaffin cells, although with a different dose dependence than wild-type CIRL and its N-terminal deletion mutant. Thus the signaling domains of CIRL are not critically important for the stimulation of exocytosis in intact chromaffin cells by α-latrotoxin.

α-Latrotoxin, a potent natural stimulator of secretion from neurons and secretory cells, has two structurally and pharmacologically distinct classes of high affinity receptors (1). The calcium-dependent receptor of α-latrotoxin or neuromin Ia is a large (160–220 kDa) cell surface membrane protein existing in multiple isoforms (2, 3). It has one transmembrane segment and structurally resembles cell adhesion proteins (4). A second high affinity receptor is the calcium-independent receptor of α-latrotoxin (CIRL) (5, 6). CIRL is thought to be more important for α-latrotoxin effects in neurons than neuromin Ia because α-latrotoxin can stimulate neurotransmitter release from neurons in Ca^{2+}-free media (7, 8). CIRL, also called latrophilin, belongs to a family of closely related orphan G protein-coupled receptors (GPCRs) homologous to the secretin receptor family (9, 10). In this family of three closely homologous proteins, CIRL-1 is a brain-enriched high affinity α-latrotoxin receptor, whereas CIRL-2 is a ubiquitously expressed low affinity receptor of the toxin (11).

There is ample evidence that α-latrotoxin receptors are critically important for the effects of α-latrotoxin (1, 12, 13). However, the mechanism of signaling downstream of the receptors is not known. The heptahelical structure of CIRL suggests its function as a regulator of a G protein pathway. However, no coupling of CIRL to any G protein has been convincingly shown. Moreover, no direct data are currently available to prove that α-latrotoxin acts as an agonist or antagonist of its receptors.

To analyze the structural requirements for α-latrotoxin binding and α-latrotoxin stimulatory function, we generated three series of CIRL deletion mutants. Soluble fragments of the extracellular region of CIRL were used to map the α-latrotoxin-binding site. On the basis of this information, N-terminally truncated membrane-bound forms of CIRL were produced, which were shown to retain high affinity α-latrotoxin binding activity. Finally, deletions in the COOH-terminal region of CIRL were produced to remove the domains potentially involved in receptor signaling. The constructs lacking the CIRL cytoplasmic tail and six of its seven transmembrane segments appeared to be fully functional in terms of α-latrotoxin binding, Ca^{2+} influx in HEK293 cells, and coupling of the toxin to secretion in transfected chromaffin cells. Our data suggest that...
G protein-mediated signaling is not critically important for the α-latrotoxin-stimulated secretion in chromaffin cells.

**Experimental Procedures**

α-Latrotoxin was purified from lyophilized black widow spider glands and radioactively labeled with 125I using the chloramine T procedure. The toxin was immobilized on BrCN-Sepharose as described (2).

**Soluble Deletion Mutants of the Extracellular Region of CIRL**—The pCDR7N construct encoding the extracellular region of CIRL (residues 1–856) with COOH-terminal His9 tag was described previously (9). The pCDR120 construct encoding the p120 subunit of CIRL precisely was prepared by ligating the AgeI/XbaI-digested PCR product with primers ACATCTAGGAGTGTCGAGCATTTGGTA and ACAAGGCCAGCGCGCACAACACATCAAGCAGAACGACGC on the 87-7 CIRL cDNA clone as a template into pCDR7 plasmid cut with AgeI/XbaI. The structure of the PCR-derived region of the final plasmid was verified by sequencing. The recombinant DNA fragments encoding other deletion mutants were prepared by high fidelity PCR with Pfu polymerase and synthetic oligonucleotide primers containing SfiI (sense) or XbaI (antisense) restriction sites. The expression constructs were prepared by ligating the SfiI/XbaI-digested PCR products into SfiI/XbaI-digested pSecTag plasmid (Invitrogen) in frame with the His9 tag. The constructs encoded the following residues of CIRL: pSSTR7-1, residues 25–598; pSSTR7-2, residues 25–856; pSSTR7-3, residues 25–631; pSSTR7-4, residues 25–705; pSSTR7-5, residues 25–770; pSSTR7-6, residues 128–856; pSSTR7-7, residues 538–936; pSSTR7-8, residues 467–950; and pSSTR7-9, residues 185–856. The plasmids were transfected into COS-7 cells using the LipofectAMINE method according to Life Technologies, Inc. protocol. After 3 days, the conditioned media and cells were harvested and analyzed for the presence of the recombinant protein by precipitation with nickel-agarose followed by Western blotting with anti-p120 antibody.

**N-terminal Deletion Mutants of CIRL**—To generate the N-terminal deletion mutants anchored to the membrane-bound fragment of CIRL, AgeI/XbaI-digested pSSTR7-6, -7, -8, and -9 plasmids were ligated with a DNA fragment of 3,060 base pairs obtained by digesting pCDR7 with AgeI/XbaI. This insert encoded a short COOH-terminal region of p120 and the entire p85 subunit. These constructs encoded the following residues of CIRL: pSSTR7-6M, residues 128–1471; pSSTR7-7M, residues 394–1471; pSSTR7-8M, residues 467–1471; pSSTR7-9M, residues 538–1471. COS cells were transfected with these plasmids, harvested in 3 days, and analyzed for α-latrotoxin binding activity as described below.

**Purification of the Recombinant Extracellular Domain of CIRL and the Analysis of Its Affinity to α-Latrotoxin**—COS cells were transfected with an expression plasmid pCDR7N encoding the entire N-terminal extracellular region of CIRL (1–857) residues with a His9 tag at the COOH terminus. In 2 days, the cell media were collected, and 10 ml of media were incubated with 300 μM imidazole in the same buffer. The binding activity of the eluted proteins was measured in the presence of 100 nM cold toxin.

**RESULTS**

**Domain Structure of CIRL**—Computer-assisted analysis of the CIRL protein sequence reveals a number of distinct structural domains (Fig. 1). A central region of CIRL (residues 850–1100) shows significant homology to the members of the secretin family of GPCRs (9). According to the hydrophobicity plot of CIRL, this region contains seven long hydrophobic stretches, a hallmark of GPCRs. In GPCRs, these hydrophobic sequences are α-helical rods that form a compact oval-shaped integral transmembrane cluster (15). Similar to other GPCRs, three intracellular and three extracellular hydrophilic loops in between transmembrane helices can be identified in CIRL.

The intracellular COOH-terminal region of CIRL (residues 1100–1471) is unusually large for an average GPCR. It contains a pair of vicinal Cys residues typical for the GPCR palmitoylation site and several proline reach clusters. It has no significant homology to any known protein except for two other members of the CIRL family, CIRL-2 and -3 (11).

In contrast, several domains of the extracellular N-terminal region of CIRL show significant homology with various receptor and nonreceptor proteins. The signal peptide sequence is followed by a cysteine-rich domain (residues 30–120) homologous to sea urchin egg p-galactoside-specific lectin (GenBank number P22031) and plant β-galactosidase (GenBank number Z99708). The same domain is found in a Caenorhabditis elegans homolog of CIRL (GenBank number Z54306). The adjacent domain (residues 120–400) is similar to olfactomedin (GenBank number AF028740), a major structural block in the extracellular matrix of the olfactory neuroepithelium and several structurally related proteins including the pancortin family (GenBank number Q62609, D78264, D78262, Q99784, AB006688, AF049796, AF035301, Q99772, AF039869). Their physiological role is unclear.

The next structural domain (residues 400–470) is enriched in Ser, Thr, and Pro residues and shows insignificant homology to mucin and other Pro-rich proteins. We will therefore refer to this domain as the NTP domain. The NTP domain is followed by a short region (residues 470–540) with a Cys-rich motif CX10–11WX4–6CX9 CX4–6CX10–11 identified by V-Blast search in the extracellular domains of GPCRs, which belong to the secretin receptor family. Interestingly, in “normal” receptors of this family (e.g., vasoactive intestinal peptide receptor, pituitary adenylate cyclase-activating polypeptide receptor, secretin receptor, calcitonin receptor, corticotropin-releasing factor receptor, growth hormone-releasing hormone receptor, glucagon-like peptide receptor, etc.) this motif is located very close to the transmembrane core. In the CIRL family, this motif is found in a number of other large orphan GPCRs (GenBank numbers U39848, Z54306, D87469, AB011529, AB011528, AB005297, AB011536, AB011122). This motif is located several hundred amino acid residues from the membrane segments, and therefore we may assume that the large orphan receptors represent a separate subfamily within the family of the secretin receptor.

The region between residues 541 and 800 has low homology

**Interaction of α-Latrotoxin with CIRL**

3591
to brain-specific angiogenesis inhibitor-3 (GenBank number AB005299), another large orphan GPCR that among all known large GPCRs is most similar to the members of the CIRL family. The recently discovered brain-specific angiogenesis inhibitor family was implicated in tumor angiogenesis regulation; brain-specific angiogenesis inhibitor-1 expression is regulated by p53 (16, 17).

Finally, in the COOH terminus of p120 immediately adjacent to the putative site of CIRL proteolysis a novel structural motif is found that is characteristic for about a dozen large orphan GPCRs of the secretin receptor family (GenBank numbers U39848, U76764, P48960, Q61549, Q14246, AC004262, D87469, AB011529, AB011528, AB011536, AB005297, X81892, AB005298, AF006014, AB011122). We propose to name this motif GPS for GPCR proteolysis site. The characteristic feature of the GPS domain is a cysteine signature including CXXC, two additional cysteine residues, and two tryptophan residues at fixed positions. When conserved residues of the GPS motif were mutated, CIRL could no longer be proteolyzed endogenously.2

Mapping the \(\alpha\)-Latrotoxin-binding Site in CIRL—We showed earlier that the recombinant N-terminal extracellular region of CIRL binds efficiently to immobilized \(\alpha\)-latrotoxin (9). To identify the \(\alpha\)-latrotoxin binding domain(s) of CIRL more precisely, we have generated a series of deletion mutants of its extracellular region. The desired DNA fragments were prepared by high fidelity PCR with synthetic oligonucleotide primers that were designed according to the putative domain borders of the extracellular region of CIRL (Fig. 2). The PCR fragments were cloned into a pSecTag eucaryotic expression plasmid (Invitrogen), which contained a signal peptide sequence that allowed extracellular secretion of soluble proteins and a COOH-terminally fused His6 tag that allowed easy purification of the recombinant protein.

The resulting constructs were transfected into COS cells. The cells and media were harvested and analyzed for the presence of recombinant proteins by the adsorption onto \(\alpha\)-latrotoxin-Sepharose followed by Western blotting with anti-p120 antibody. Among tested constructs, pCDR-120, pCDR7N, and pSTR7-2, -6, -7, and -20 were expressed and secreted in the medium. Proteins encoded by pSTR7-1, -3, -4, -9, and -16 were expressed well but accumulated inside the cells (data not shown). It is interesting to note that most of the secreted proteins were N-terminally truncated, whereas most of the nonsecreted deletion mutants were COOH-terminally truncated. This finding raises a possibility that the extracellular region of CIRL contains in its COOH-terminal part a signal sequence that regulates intracellular sorting and trafficking of the protein.

To analyze the \(\alpha\)-latrotoxin binding activity of the recombinant proteins, the conditioned media or cell extracts were adsorbed onto \(\alpha\)-latrotoxin-Sepharose followed by Western blotting with anti-p120 antibody. Among tested constructs, pCDR-120, pCDR7N, and pSTR7-2, -6, -7, and -20 were expressed and secreted in the medium. Proteins encoded by pSTR7-1, -3, -4, -9, and -16 were expressed well but accumulated inside the cells (data not shown). It is interesting to note that most of the secreted proteins were N-terminally truncated, whereas most of the nonsecreted deletion mutants were COOH-terminally truncated. This finding raises a possibility that the extracellular region of CIRL contains in its COOH-terminal part a signal sequence that regulates intracellular sorting and trafficking of the protein.

To analyze the \(\alpha\)-latrotoxin binding activity of the recombinant proteins, the conditioned media or cell extracts were adsorbed onto \(\alpha\)-latrotoxin-Sepharose followed by Western blotting with anti-p120 antibody (Fig. 3). The constructs pCDR-120, pCDR7N, and pSTR7-2, -5, -6, -7, and -20 specifically interacted with the toxin, whereas pSTR7-1, -3, -4, -9, and -16 did not. The shortest N-terminally truncated mutant that still bound the toxin was pSTR7-7. A shorter construct pSTR7-9 (residues 538–856) failed to interact with the toxin. The analysis of \(\alpha\)-latrotoxin binding activity of COOH-terminally truncated mutants demonstrated that the downstream border of the toxin-binding site may be located close to the membrane.

---

2 V. Krasnoperov, K. Ichtchenko, and A. G. Petrenko, manuscript in preparation.
The pCDR7N protein, which is the entire extracellular region of CIRL (residues 1–856) and recombinant p120 (residues 1–837, construct pCDR-120), bound to the toxin quite well. A shorter protein pSTR7-5 (residues 25–770) bound to α-latrotoxin much less efficiently, whereas pSTR7-4 (residues 25–705) did not interact with the toxin at all. Together, these data suggest that the residues critical for α-latrotoxin binding are located in the COOH-terminal half of p120 with a significant site of interaction around residue 770.

Soluble Fragments of the Extracellular Domain of CIRL Are Low Affinity α-Latrotoxin-binding Proteins—To further analyze the interaction of α-latrotoxin with the extracellular domain of CIRL and its fragments, we tested the ability of these soluble proteins to inhibit the binding of iodinated α-latrotoxin to brain membranes. Surprisingly, no significant inhibition was detected with any protein tested including the entire uncleaved extracellular domain encoded by pCDR7N, p120 (these two constructs were not based on PCR-generated sequences), and their fragments (data not shown). The concentration of CIRL fragments in the binding mixtures was typically in the range of 20–60 nM, whereas the concentration of labeled α-latrotoxin was 2 nM.

Because these same soluble CIRL fragments bound quite well to immobilized α-latrotoxin, we assumed that they were α-latrotoxin-binding proteins. However, their affinity to the toxin was lower than the affinity of endogenous CIRL, and therefore they could not compete effectively in the concentration range tested. To estimate the affinity of p120 and its deletion mutants to α-latrotoxin, we used the solid-phase assay developed earlier for the analysis of α-latrotoxin binding activity of detergent-solubilized CIRL (5). p120 solution was applied to the bottoms of 96-well plates by drying at ambient temperature. After washing and blocking, solutions with various concentrations of 125I-α-latrotoxin were added to the wells. Following multiple washes, the absorbed labeled toxin was eluted...
with SDS-containing buffer and counted for radioactivity. Scatchard plot analysis revealed that the affinity of α-latrotoxin binding to p120 was about 25 nM, which was approximately 2 orders of magnitude lower than the affinity of CIRL binding (data not shown).

High Affinity Binding of α-Latrotoxin to N-terminally and COOH-terminally Truncated Membrane-associated CIRL—Low affinity interaction of α-latrotoxin with soluble p120 raised the possibility that the formation of high affinity complex requires participation of p85. To test this, we recombined DNA fragments encoding soluble deletion mutants with the sequence of p85 so that the resulting constructs encoded N-terminally truncated forms of CIRL. In the 5-region of pSTR7-6, -7, -8, and -9, a fragment of CIRL cDNA was subcloned, which restored the junction of p120 and p85 identically to wild-type CIRL. The resulting expression constructs encoded the following residues of the CIRL protein sequence: pSTR7-6M, residues 128-1471; pSTR7-7M, 394-1471; pSTR7-8M, 467-1471; pSTR7-9M, 538-1471. The analysis of the transfected COS cells demonstrated that all mutants except pSTR7-9M bound to α-latrotoxin specifically (Fig. 4A). This result was in good agreement with the analysis of soluble deletion mutants of CIRL and suggests that lectin-like, olfactomedin-like, and STP (mucin-like) domains in the N-terminal half of p120 are not important for α-latrotoxin binding.

We further quantitated the α-latrotoxin binding activity of pSTR7-7M and pSTR7-8M, the two shortest α-latrotoxin-binding mutants, by Scatchard plot analysis. It appeared that both membrane-bound deletion mutants interacted with α-latrotoxin with the same high affinity as wild-type CIRL (Fig. 4, B and C). We can therefore conclude that the primary α-latrotoxin-binding site is located in the COOH-terminal half of p120. However, the high affinity interaction requires complexing of p120 with membrane-bound p85. Because the plasmids for membrane-bound mutants were prepared on the basis of the constructs encoding soluble CIRL fragments, this experiment also demonstrates that the low affinity of the recombinant soluble CIRL fragments was not because of a PCR or cloning artifact.

To test the importance of different regions of p85 in the interaction with α-latrotoxin, we generated two mutants of CIRL with deleted portions of the p85 subunit by introducing stop codons (Fig. 5). The first mutant pCDR-7TMR did not contain most of the large COOH-terminal cytoplasmic tail of p85. In the second mutant pCDR-1TMR, the N-terminal region of p85 with only one transmembrane segment was preserved. It appeared that both deletion mutants expressed very well and both showed α-latrotoxin binding activity (Fig. 6A). The Scatchard plot analysis of the one transmembrane mutant demonstrated high affinity binding indistinguishable from the wild-type receptor (Fig. 6B). These results rule out the involvement of the extracellular loops and the cytoplasmic tail of p85 in the stabilization of the α-latrotoxin complex with p120.

Deletion Mutants of CIRL Are Functional in Coupling CIRL to Exocytosis—We determined whether deletion constructs that possessed high affinity α-latrotoxin binding (pCDR-1TMR, pCDR-7TMR, and pCDR-7) supported α-latrotoxin-stimulated calcium influx when expressed in HEK293 cells (Fig. 7). All three proteins increased the uptake of 45Ca2+, although the pCDR-1TMR mutant was less effective than pCDR-7TMR, pCDR7-8, or CIRL itself and had little effect at 50 pM α-latrotoxin. The data indicate that the COOH-terminal part of CIRL is not required in mediating the effects of α-latrotoxin on calcium permeability and suggest that high affinity α-latrotoxin binding is sufficient for calcium influx.

We noted that at higher concentrations of α-latrotoxin cells transfected with a control plasmid (neo) or nontransfected cells (not shown) also exhibited some α-latrotoxin-stimulated 45Ca2+ influx. This increase in calcium permeability is probably because of the α-latrotoxin interaction with an endogenous HEK293 cell protein, because it is completely blocked by preincubation of the cells with concanavalin A (data not shown).

Transient expression of CIRL in chromaffin cells increases their sensitivity to α-latrotoxin (9). We asked whether the increase in calcium permeability seen with pCDR-1TMR, pCDR-7TMR, and pCDR7-8 was coupled to the secretory response in chromaffin cells. Because the transfection efficiency of primary cultures is low, the various mutants were co-expressed with human growth hormone, which is stored in secretory granules and serves as a reporter for secretion from the transfected cells. Each of the three constructs increased the sensitivity of transfected cells to low concentrations of α-latrotoxin (2.5 and 10 pM), and the magnitude of the effect was similar to that of wild-type CIRL (Fig. 8A). Catecholamine release (a measure of secretion from all the cells, the majority of which were not successfully transfected) was similar for all
the groups (Fig. 8B). We conclude that the COOH-terminal part of CIRL is unnecessary in mediating α-latrotoxin-stimulated secretion from intact chromaffin cells. Interestingly, at higher concentrations of α-latrotoxin, some inactivation of the secretory response was seen with CIRL and pCDR7-8 that did not occur with the COOH-terminal deletion mutants 1TMR and 7TMR, raising the possibility that the COOH-terminal cytoplasmic tail of CIRL plays an additional role in modulating secretion.

**DISCUSSION**

α-Latrotoxin acts extracellularly by binding to endogenous membrane receptors that belong to the neurexin and CIRL families. The formation of receptor-toxin complexes is followed by cation influx through α-latrotoxin-induced channels and by as yet unidentified signaling that eventually results in massive spontaneous exocytosis. Part of the effects of α-latrotoxin can be explained by Ca$^{2+}$ entry through the toxin-induced pores. However, the toxin is also active when applied in nominally Ca$^{2+}$-free buffers (7, 8, 18) or when cation fluxes are controlled, e.g. in permeabilized cells (14, 19). A possible explanation of the calcium-independent effect of α-latrotoxin is that it activates its membrane receptors, which results in intracellular signaling, through a G protein-linked pathway (19, 20).

To analyze the interaction of α-latrotoxin with CIRL and subsequent functional effects, we have generated a set of CIRL deletion mutants that were examined in binding experiments, $^{45}$Ca$^{2+}$ influx assays in HEK293 cells and secretion experiments in chromaffin cells. We found that 1) small segments of the extracellular and membrane domains of CIRL are required for high affinity binding to latrotoxin and for functional effects of latrotoxin, and 2) CIRL-coupled G protein signaling is not critically important for α-latrotoxin-stimulated Ca$^{2+}$ influx in HEK293 cells or secretion from chromaffin cells. The evidence in support of these conclusions is discussed below.

**High Affinity Binding of Latrotoxin Requires a Short Extracellular Segment and the First Transmembrane Domain—**

CIRL consists of two noncovalently bound subunits, p120, which is extracellular, and p85, which contains seven transmembrane segments and the COOH-terminal cytoplasmic domain. The analysis of a series of soluble recombinant fragments of CIRL by precipitation with α-latrotoxin-agarose showed that the α-latrotoxin-binding site lies within the COOH-terminal half of p120. However, none of the recombinant soluble fragments of CIRL (including p120 and the nonprocessed full-size extracellular region, pCDR7N) that bound effectively to immobilized α-latrotoxin were able to compete with the binding of α-latrotoxin to endogenous CIRL in brain membranes. The Scatchard plot analysis of the α-latrotoxin binding activity of the full-length extracellular region indicated that its affinity was about 2 orders of magnitude lower than the affinity of CIRL.

In contrast, two N-terminally truncated fragments of CIRL, which contained p85 in addition to the α-latrotoxin binding domain of p120, bound the toxin with the same high affinity as wild-type CIRL. The extracellular loops of p85 are apparently not important for the stabilization of the complex with the toxin because a deletion mutant, which contained only the first transmembrane segment, bound to α-latrotoxin with high affinity.

Therefore we propose that the interaction of α-latrotoxin with CIRL consists of two sequential steps. At first, α-latrotoxin binds with medium affinity to the extracellular region of CIRL. Following this binding, the toxin interacts with the first transmembrane segment of CIRL, with the membrane lipids, or with both and penetrates into the lipid bilayer as a result. This second step increases affinity of the interaction and may require a longer time, which would explain a known delay in α-latrotoxin effects within a minute after its application.

**Only a Single Transmembrane-spanning Domain Is Required to Support Latrotoxin-induced Ca$^{2+}$ Influx and Secretion—**

CIRL deletion mutants that bound α-latrotoxin with high affinity were also effective in coupling α-latrotoxin to calcium influx into HEK293 cells and exocytosis in chromaffin cells,
although the 1TMR mutant was noticeably less effective than wild-type CIRL and other mutants in the $^{45}$Ca$^{2+}$ uptake assay. Most importantly, a mutant receptor lacking six of seven transmembrane segments was similar to wild-type CIRL in the secretion experiments. Because this mutant receptor would be unable to activate G proteins, these experiments indicate that the receptor does not mediate the stimulatory effect of $\alpha$-latrotoxin in intact chromaffin cells by direct coupling to G proteins.

The shape of the dose-effect curves for overexpressed wild-type CIRL and its N-terminally truncated mutant was significantly different from those of the COOH-terminal mutants (Fig. 8A). The dose dependence of the $\alpha$-latrotoxin-stimulated exocytosis in chromaffin cells via either endogenous receptors, overexpressed CIRL, or its N-terminal mutant was bell-shaped (14) (Fig. 8). In contrast, for both COOH-terminally truncated mutants, the dose dependence did not show “inactivation” at higher concentrations of $\alpha$-latrotoxin. It is therefore possible that $\alpha$-latrotoxin binding to CIRL results in multiple effects, one of which is dependent upon the integrity of the COOH terminus of the p85 subunit.

What may be the physiological importance of CIRL? Because it serves to target $\alpha$-latrotoxin, a potent stimulator of secretion, it is likely that this receptor is positioned appropriately for the regulation of exocytosis. CIRL has been shown to co-purify with syntaxin, a t-SNARE (9). In permeabilized chromaffin cells overexpression of CIRL inhibits Ca$^{2+}$-stimulated secretion (14). Together, these findings suggest that CIRL may serve as a physiological regulator of exocytosis. Because of the presence of cell adhesion structural modules in its extracellular domain, it is possible that direct physical contacts of cells modulate secretion via CIRL-mediated signaling.