Isolation and Biochemical Characterization of a Ca\(^{2+}\)-independent α-Latrotoxin-binding Protein*

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α-Latrotoxin, a black widow spider neurotoxin, can bind to high affinity receptors on the presynaptic plasma membrane and stimulate massive neurotransmitter release in the absence of Ca\(^{2+}\). Neurexins, previously isolated as α-latrotoxin receptors, require Ca\(^{2+}\) for their interaction with the toxin and, thus, may not participate in the Ca\(^{2+}\)-independent α-latrotoxin activity. We now report the isolation of a novel protein that binds α-latrotoxin with high affinity in the presence of various divalent cations (Ca\(^{2+}\), Mg\(^{2+}\), Ba\(^{2+}\), and Sr\(^{2+}\)) as well as in EDTA. This protein, termed here latrophilin, has been purified from detergent-solubilized bovine brain membranes by affinity chromatography on immobilized α-latrotoxin and concentrated on a wheat germ agglutinin affinity column. The single polypeptide chain of latrophilin is N-glycosylated and has an apparent molecular weight of 120,000. Sucrose gradient centrifugations demonstrated that latrophilin and α-latrotoxin form a stable equimolar complex. In the presence of the toxin, anti-α-latrotoxin antibodies precipitated iodinated latrophilin, whose binding to immobilized toxin was characterized by a dissociation constant of 0.5–0.7 nM. This presumably membrane-bound protein is localized to and differentially distributed among neuronal tissues, with about four times more latrophilin expressed in the cerebral cortex than in the cerebellum; subcellular fractionation showed that the protein is highly enriched in synaptosomal plasma membranes. Our data suggest that latrophilin may represent the Ca\(^{2+}\)-independent receptor and/or molecular target for α-latrotoxin.

First, studies in neuronal and PC12 cells demonstrate that the toxin-receptor interaction does not require Ca\(^{2+}\), although the removal of Ca\(^{2+}\) appreciably decreases the binding (5, 6). This result is best explained by the existence of two classes of LTX receptors, Ca\(^{2+}\)-dependent and independent, both presumably active when Ca\(^{2+}\) is present. These receptor types possess similar high affinities to LTX (7) but display different toxin binding properties (e.g., the Ca\(^{2+}\)-independent binding is more sensitive to high salt) (8). Furthermore, in at least one PC12 cell line only the Ca\(^{2+}\)-independent binding was detectable (7), indicating that the heterogeneous LTX receptors are probably differentially regulated and, thus, may also be structurally different.

Ca\(^{2+}\) is also not essential during the second phase of the toxin action and can be readily substituted for by a variety of divalent cations (5). The action of LTX in the absence of Ca\(^{2+}\) depends on the nature of the divalent cation present, being quite slow in Mg\(^{2+}\). Apparently, under these conditions the toxin can set off neurotransmitter release by an alternative mechanism, which bypasses Ca\(^{2+}\) and might be regulated by other physiological stimuli (3, 9, 10). Moreover, even the requirement for divalent cations has been challenged by observations that the toxin causes release in EDTA at frog neuromuscular junctions in hypertonic solutions (11) and in rat cerebrocortical synaptosomes (12). Further evidence in support of the dual character of the toxin activity was provided by the use of a monoclonal antibody that binds to LTX and completely blocks its channel-forming activity but does not abolish the toxin-induced release (13). These data indicate that the LTX receptor may act as an active conductor of the toxin stimulatory effect rather than a passive acceptor site to help LTX form cation channels.

Finally, LTX, whose receptors have been found exclusively in synaptic endings (2), induces massive vesicle exocytosis at the active zones (14, 15). This effect is more extensive than release in high K\(^{+}\) (8, 16) but is apparently highly localized to the presynaptic membrane as it can happen without an increase in the intracellular Ca\(^{2+}\) concentration or bulk redistribution of cytoplasmic Ca\(^{2+}\) (16). Therefore, the LTX receptors are probably located in proximity to the neurosecretion machinery and may represent important control proteins for the release process.

These considerations warranted significant interest in the nature of the LTX receptors. Several attempts have been made to characterize and purify these proteins (17–19), and the isolation of a receptor complex using a LTX affinity column was reported (19). High molecular weight components of that complex (M\(_{r}\) 200,000 and 160,000) were shown to bind the toxin in a Ca\(^{2+}\)-dependent manner (19, 20). These polypeptides proved to be members of a family of heterogeneous neuronal cell-
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surface proteins, termed neurexins (21). Recombinant neurexin Iα was found to bind LTX only in the presence of Ca\textsuperscript{2+} (22). Thus, neurexins may not represent the Ca\textsuperscript{2+} -independent LTX receptors that have not been characterized yet. Herein, we report the purification from detergent-solubilized bovine brain membranes of a neuronal protein that binds LTX in vitro with high avidity in the absence as well as in the presence of Ca\textsuperscript{2+}. Partial sequencing of this protein confirmed its novelty, 2 and to reflect the protein’s strong affinity toward LTX, we refer to it here as latrophilin.

EXPERIMENTAL PROCEDURES

Isolation of the α-Latrotoxin-binding Protein—LTX was purified from the venom of Latrodictus tredecimguttatus (18) and coupled to CNBr-activated Sepharose 4B (Pharmaecia Biotech Inc.) as described previously (19). The resulting column contained 2 mg of immobilized toxin/4 ml of gel. Bovine cerebral cortex and liver membranes (or rat total brain and synaptosomal membranes) (23) were solubilized in 2% Triton X-114, washed extensively, and treated with 20% (v/v) of 5 mM EDTA (see Methods). To specifically immunoprecipitate bled two weeks after the fourth injection.

Immunobeads were prepared by successively and chloramine T as described (18). To specifically immunoprecipitate

Batch-incubated with the LTX-Sepharose for 2 h. The gel was then washed in a column with 60 volumes of 0.5 M NaCl in TE containing 0.6% CHAPS (TE-CHAPS). In experiments where the influence of di-

valent cations was studied, the buffers during the incubation and wash-

steps contained 2 mM of CaCl\textsubscript{2}, BaCl\textsubscript{2}, SrCl\textsubscript{2}, or MgCl\textsubscript{2} instead of 

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2 mM of CaCl\textsubscript{2} (TE-CHAPS). In experiments where the influence of divalent cations was studied, the buffers during the incubation and washing steps contained 2 mM of CaCl\textsubscript{2}, BaC

4 l of the anti-LTX-antiserum and 5

5 l of affinity-purified protein (from the LTX-Sepharose) were added to a micro-column of the WGA-Sepharose 4B and the column was washed with 10 ml of 0.1 M NaCl in TM buffer (4). After extensive washing with buffer, glycoproteins were desorbed from the column with 100 ml of 0.5 M Na-acetylgalactosamine in TSE and analyzed as above.

Other Methods—Protein concentrations were determined by the method of Lowry (24). Phase separation of latrophilin (eluted from the LTX column in Triton X-114) and \textsuperscript{125}I-LTX (treated with the detergent prior to partitioning) was established as described (25). SDS-PAGE analysis of protein fractions was performed according to Laemmli (26) in 8% constant porosity and 4–20% gradient gels, which were calibrated using protein standards (Bio-Rad). After electrophoresis, gels were stained with Coomasie or silver; otherwise proteins were transferred onto polyvinylidene difluoride membrane (Millipore), immunostained, and visualized by ECL as specified by the manufacturer (Amersham Corp.).

RESULTS

α-Latrotoxin Affinity Chromatography—Solubilized bovine brain membrane proteins were affinity chromatographed on a LTX column in the presence of 5 mM EDTA. When choosing the desorption conditions, we exploited the fact that considerably lower binding of LTX to membrane preparations was observed in high NaCl (5). After extensive washing with the loading buffer (Fig. 1A, lane 3), the column was eluted with high salt. A single protein with a molecular weight similar to that of LTX (M\textsubscript{r} about 120,000) was found in the eluate (lane 4). This protein not only came off the column and was completely eluted by large volumes of high salt buffer (2 M NaCl). The slow rate of elution suggested that the M\textsubscript{r} 120,000 protein was strongly but reversibly bound to the column and desorbed because the equilibrium of the binding reaction was reversed by the continuous addition of a large excess of fresh high salt buffer. The gel was further washed with a denaturing buffer to ascertain that no other proteins were retained by the column under these conditions. 1% SDS was able to elute only some residual M\textsubscript{r} 120,000 protein from the column (Fig. 1A, lane 6).

We initially concentrated the eluted M\textsubscript{r} 120,000 protein by ultrafiltration. However, this procedure was inconvenient for routine processing of large volumes and often resulted in the precipitation of the M\textsubscript{r} 120,000 protein on the filter. Therefore, another step of affinity chromatography was tested, in which a WGA matrix was used. This was tried because of the known fact that WGA potently inhibits LTX binding to its receptor in synaptosomes (18). The M\textsubscript{r} 120,000 protein bound quantitatively to the WGA column and eluted in a small volume of 100 mM N-acetylgalactosamine (Fig. 1A, lane 5). This stage allowed us to efficiently concentrate the affinity-purified protein without loss of toxin-binding activity, and this doubly affinity-purified protein was used in all subsequent experiments.

To prove that the M\textsubscript{r} 120,000 protein adsorbed on the affinity

2 Yu. Ushkaryov, unpublished observation (based on peptide sequencing and partial cloning).
Previously, the isolation of neurexins was performed on LTX columns in buffers supplemented with Ca\(^{2+}\) (17, 19). It was important now to show that under the same conditions the M\(_r\) 120,000 protein also binds to immobilized LTX. Consistent with its high affinity to LTX and with the previous observations (19, 20), the M\(_r\) 120,000 protein was indeed found in the eluate after chromatography had been carried out in 2 mM Ca\(^{2+}\) (Fig. 1B). As expected, α-neurexins (known as the α- and α’-subunits of the “receptor complex” (19)) also eluted from the column but were absent from any fractions during chromatography in EDTA (Fig. 1, A and B). In fact, neurexins did not interact with the LTX-column even in the presence of Ba\(^{2+}\), Sr\(^{2+}\), or Mg\(^{2+}\), divalent cations known to support the toxin-elicited neurosecretion (Fig. 1B, left panel). In contrast, the M\(_r\) 120,000 protein could be affinity purified to high homogeneity under all conditions tested.

The high degree of purification of the 120-kDa protein allowed the production of a specific antiserum. Upon immunoblotting with the antiserum (Fig. 1A, lower panel), the M\(_r\) 120,000 protein exhibited no cross-reactivity with LTX or neurexins (Fig. 1, A and B). As partial sequencing of the protein revealed no homologies in the current data bases (not shown), it was tentatively called latrophilin.

**Interaction of α-Latrotoxin with Latrophilin**—The very gradual elution of latrophilin from the LTX column in 2 mM NaCl indicated a high affinity interaction. However, it proved difficult to quantitatively separate the two high molecular weight proteins from their complex in solution. Thus, the strength and specificity of binding were characterized by methods based on immobilization of one of the interacting proteins. In one of these approaches, an anti-LTX antibody immunoprecipitated \(^{125}\)I-latrophilin when LTX was present (Fig. 2A, left panel). BSA or solubilized liver membranes, when added, had no effect on immunoprecipitation. In the absence of either the toxin or the antibody, latrophilin did not specifically interact with Immunobeads. A large excess of unlabeled latrophilin competed with the iodinated protein for LTX, suggesting a direct and specific interaction of the two molecules. This was further confirmed by a nitrocellulose filter binding assay, which only worked when the toxin (Fig. 2A, right panel), but not latrophilin was immobilized on the filter. The pre-adsorption of latrophilin on nitrocellulose resulted in its inactivation (not shown).

Because of the high background, the nitrocellulose assay did not allow us to carry out quantitative measurements. Therefore, the affinity of the two proteins was determined by measuring the binding of radiolaabeled latrophilin to LTX attached to Immunobeads (as detailed under “Experimental Procedures”) or to Sepharose (the affinity column). Respective dissociation constants (K\(_d\)) of the \(^{125}\)I-latrophilin-LTX complex were 0.75 and 0.54 m\(\text{M}\) (Fig. 2B). These very similar values are characteristic of high affinity binding and fall within the range of K\(_d\) values reported for the LTX receptors (3).

To determine an approximate size of the LTX-latrophilin complex(es) and the stoichiometry of its constituents, these proteins were centrifuged through sucrose density gradients either individually or in different molar combinations. Loaded alone, LTX travelled as a concise peak (Fig. 3A, panel a) with an apparent molecular weight of about 120,000. In a separate gradient, latrophilin migrated as a peak with a buoyant density similar to that of LTX; however, some dimerization of latrophilin apparently occurred under these conditions (b). In the presence of a large molar excess of its counterpart, each protein markedly changed its migration pattern (Fig. 3A, c and d). The apparent size of the complex was greater than that of the individual components and peaked around 250,000, indicating that the complex consisted of one molecule of each protein.

**Matrix specificity and purification.** Two control columns were prepared and utilized in affinity chromatography: 1) regular LTX-column denatured with SDS and β-mercaptoethanol and 2) CNBr-Sepharose coupled with BSA instead of LTX. Upon chromatography of solubilized bovine brain membranes, the eluate from the denatured toxin column (Fig. 1A, lane 7) showed no trace of the M\(_r\) 120,000 protein. The BSA column produced the same result. In another control experiment, we chromatographed solubilized bovine liver membranes on a fresh portion of LTX column (lane 8). No M\(_r\) 120,000 band was observed in the liver eluate. The results of control experiments suggest that this protein is expressed in the brain and interacts specifically with the toxin only in its native conformation.

![Figure 1](http://www.jbc.org/)
Both proteins trailed toward the bottom of the gradient in parallel, indicating that some aggregation of the complexes was possible. These two proteins interacted specifically, since unlabeled toxin totally competed $^{125}$I-LTX off the complex (e). BSA, centrifuged in a mixture with LTX or latrophilin, or both proteins together, did not change its own position (fraction 14, not shown) nor did it displace either of the proteins from the complex. Silver staining of gradient fractions separated by SDS-PAGE demonstrated that both proteins were present in equal amounts in the complex (Fig. 3B). When one protein was used in excess, only part of it, which was involved in the complex, shifted to higher gradient densities; the rest migrated at its normal position (Fig. 3B). These results suggest that latrophilin and LTX form equimolar and saturable specific complexes.

**Properties of Latrophilin**—Treatment with glycohydrolases proved that latrophilin is glycosylated, with the relative molecular mass of the deglycosylated polypeptide chain around 95,000 (Fig. 4A). Treatment with neuraminidase produced a small change in the molecule’s electrophoretic mobility demonstrating a low degree of sialylation. Sialic acids could account for about 2–4 kDa in the apparent molecular mass of the intact protein. There was no detectable mobility shift when latrophilin was digested with O-glycosidase, indicating that there is very little, if any, O-type glycosylation present in this protein. Thus, N-attached carbohydrates contribute up to 25 kDa (or 20%) to the molecular mass of the protein. Deglycosylation produced only one species of a smaller size (Fig. 4A), allowing us to assess the purity of the latrophilin preparation. If unrelated protein(s) were present in the original band of $M_r$ 120,000, they would be highly unlikely to exhibit exactly the same deglycosylation pattern. Thus, the preparation proved homogeneous.

When analyzed by SDS-PAGE under nonreducing conditions latrophilin migrated as a band of approximately 114 kDa (Fig. 4B, left panel), which did not produce any smaller proteins, demonstrated that disulfide bridges do not link two or more protein chains in the native molecule. Interestingly, in the presence of $\beta$-mercaptoethanol latrophilin displayed an even lower mobility than in its absence. This behavior implies that latrophilin consists of a single polypeptide chain, whose intramolecular disulfide bonds render the unreduced molecule more compact than the reduced one.

Latrophilin could be purified only from brain membranes solubilized with detergent and was not extractable by salt solutions (not shown), indicating that this protein was membrane bound. To test its hydrophobic nature, we followed the redistribution of the protein between two phases formed in Triton X-114 solutions. As evident from Fig. 4C, latrophilin (unlike LTX) was preferentially partitioned into the detergent-rich phase. Thus, latrophilin is most likely a membrane-bound protein (26).

**Tissue Distribution of Latrophilin**—To evaluate the tissue...
Latrophilin is a brain-specific protein highly enriched in synaptosomal plasma membranes. Crude membranes (P₂) were prepared from bovine cerebral cortex, cerebellum, liver, kidney, and lung (left panel) or from total rat brain (right panel). These membrane preparations were solubilized and glycoproteins isolated by affinity chromatography on identical WGA columns as described under "Experimental Procedures." Aliquots (50 μg of protein) were run on an SDS-gel in parallel with 10 ng of latrophilin. Proteins were immunobotted using an anti-latrophilin antibody and visualized by enhanced chemiluminescence. Relative mobilities of standard protein markers are indicated on the left.

The sensitivity problem we exploited the ability of latrophilin to bind effectively to lectins. WGA affinity chromatography was used to enrich solubilized membrane proteins in respect of surface glycoproteins and was conducted in parallel for all tissues. Eluates from WGA columns were analyzed by Western blotting. As Fig. 5, left panel, clearly demonstrates, latrophilin is expressed only in nervous tissues and is most abundant in the cerebral cortex. What is the maximal level of latrophilin expression? Table I presents the results of a quantitative immunostaining conducted on various brain membrane preparations. In keeping with the previously published data for latrotoxin binding sites (3, 17), latrophilin content in the bovine brain cortex is about 40 ng (0.33 pmol)/mg of membrane protein. Four times less latrophilin has been found in the bovine cerebellum. The rat brain contains 28 ng (0.23 pmol) of latrophilin/mg.

How is the protein distributed at the subcellular level? To answer this question, we purified rat brain synaptosomal plasma membranes. Western blotting of this fraction demonstrated that latrophilin concentration is significantly (seven times) higher in synaptic plasma membranes than in total rat brain membranes (Fig. 5, right panel, and Table I) and reaches 200 ng (1.67 pmol)/mg of synaptosomal plasma membrane protein.

Latrophilin Purification Yields—Results of representative latrophilin purifications from various brain membrane preparations are shown in Table I. Compared with an independently determined maximal level of the protein in the brain, yields higher than 30% were routinely achieved. Homogeneous latrophilin has also been isolated from rat brain membranes and rat brain synaptosomes. Consistent with the immunostaining data, about six times more protein per mg was obtained from synaptosomes than from total brain membranes. Finally, the purification factor attained during the one-step affinity chromatography was approximately 25,000 and 4,000 when starting with brain membranes and synaptosomes, respectively.

**DISCUSSION**

α-Latrotoxin stimulates extensive neurotransmitter exocytosis from vertebrate synapses both in the presence and in the absence of Ca²⁺, provided that other divalent cations are present. During the search for LTX receptors, a family of cell-surface neuronal proteins, neurexins, has been isolated and characterized (19, 21). Of these, neurexin Iα was found to bind...
LTX in vitro in a Ca\(^{2+}\)-dependent manner (22). Our data (Fig. 1B) confirm that neurexins can only be purified on immobilized LTX when Ca\(^{2+}\) is present in the medium; EDTA completely abolishes neurexin's binding to the LTX column. Interestingly, neurexins were also undetectable in eluates upon affinity chromatography in other divalent cations (Mg\(^{2+}\), Ba\(^{2+}\), or Sr\(^{2+}\)). These ions, however, are well known to support the LTX-induced neurotransmitter release (5). Thus, when applied in Ca\(^{2+}\)-free media containing other divalents, the toxin does not act via neurexins. An important question remains as to whether neurexins propagate the LTX effect when Ca\(^{2+}\) is present. Indeed, the binding of the toxin to synaptosomes and PC12 cells is higher in Ca\(^{2+}\) than in any other cation, including Ba\(^{2+}\) and Sr\(^{2+}\) (7, 8). This has been verified in our experiments on rat synaptosomes,\(^3\) suggesting that in Ca\(^{2+}\), LTX binds both to neurexins and to the Ca\(^{2+}\)-independent receptor. However, in Ba\(^{2+}\) and Sr\(^{2+}\) the toxin causes the same strong and fast release as in Ca\(^{2+}\), in spite of the lower level of binding (shown on PC12 cells in Ref. 5 and on synaptosomes\(^3\)). Taken together, these results indicate that only the Ca\(^{2+}\)-independent receptor plays an active role in the toxin-triggered release, and that neurexins, although capable of binding LTX in the presence of Ca\(^{2+}\), do not actively participate in the toxin action under any conditions. Since no direct evidence (e.g. the neurexin gene knockout) is available at present to prove this conclusion, it remains only hypothetical, making the isolation and study of the alternative, Ca\(^{2+}\)-independent LTX receptor even more crucial.

We describe here the purification of latrophilin, a novel brain-specific protein, structurally distinct from neurexins. While accompanied by neurexins during chromatography in Ca\(^{2+}\), latrophilin was the only protein binding to the LTX column under any other conditions (Fig. 1). The following features of this protein are worth reiterating: latrophilin binds LTX with high affinity. Its dissociation constant (0.5–0.7 nM) is in good agreement with that reported for LTX binding to synaptosomes (0.1–1 nM) (5, 6, 18). Similar to LTX receptors in vivo (8, 27), latrophilin is much more abundant in the bovine cortex than in the cerebellum and is undetectable in non-neuronal tissues. Immunohistochemically determined latrophilin content in the bovine cerebral cortex is very low (0.33 pmol/mg), which compares well with the yield of the affinity-purified protein and is very close to the maximal number of LTX-binding sites (3, 17). As expected of a synaptic protein, latrophilin is enriched in synaptosomes and is even further concentrated in synaptosomal plasma membranes (Fig. 5 and Table I), indicating that it may be a cell-surface receptor. In support of this view, we demonstrate also that latrophilin is a membrane-bound glycoprotein. Finally, the specificity of latrophilin-LTX interaction is so high that it results in a 25,000-fold purification of latrophilin in one step. These and other features of latrophilin suggest that this protein is the most likely candidate for the Ca\(^{2+}\)-independent LTX receptor. If we assume that latrophilin were not the receptor, then the observed absence of binding of any other proteins to the affinity column would have had two implications: either the receptor is not a protein or it is totally inactivated by the solubilization procedure. Both statements are apparently incorrect: the protein nature of the LTX receptor has long been established (6, 8). It also seems unlikely that the mild solubilization procedure would lead to a complete inhibition of the high affinity binding. In addition, our preliminary experiments, where anti-latrophilin antibodies specifically bound to the surface of intact synaptosomes (not shown), prove the surface exposure of latrophilin in the synaptic plasma membranes. Obviously, as the current study deals only with in vitro experiments, the final evidence for the involvement of latrophilin (as well as neurexins) in the LTX binding to neuronal plasma membranes will have to be sought in vivo.

Since latrophilin binds LTX with very high specificity and affinity, it must have been present in all preparations during the previous attempts to isolate the receptor. In fact, a protein of a similar molecular weight to that of latrophilin was previously observed in eluates after LTX affinity chromatography in Ca\(^{2+}\) (19, 20, 28). That protein was considered to be the toxin itself because of its molecular weight and due to LTX bleeding from the affinity gel. On the contrary, our results prove that latrophilin is unrelated to the toxin. We also found in this study that latrophilin is inactivated upon its attachment to a surface (e.g. nitrocellulose). The use of solid-phase assays to monitor the toxin binding to immobilized receptor preparations explains why LTX interaction only with neurexin (but not latrophilin) was detected in the past (17, 19). However, when a receptor preparation, presumably containing both neurexins and latrophilin, was reconstituted in liposomes, specific binding of LTX could be measured in various divalent cations or in EDTA (29). Importantly, under all ionic conditions, the toxin formed ion-permeable channels in these receptor-bearing (but not control) liposomes. This finding further supports the idea that LTX induces ion fluxes as a result of its direct interaction with latrophilin, which may also participate in other effects of the toxin.

To summarize, two structurally different proteins are present in the brain that strongly bind latrotoxin: neurexins and latrophilin. What could be the role of each of these proteins in the toxin action? There is no definitive answer to this question yet, but the following scheme seems plausible. Both α-neurexins and latrophilin bind the toxin under physiological conditions, in the presence of Ca\(^{2+}\). Being more abundant, neurexins may create a high local concentration of LTX close to the

\(^3\) B. A. Davletov, O. G. Shamotienko, and Yu. A. Ushkaryov, manuscript in preparation.

| Species | Source of membranes\(^a\) | Latrophilin content\(^b\) | Latrophilin purified\(^c\) | Yield \(\%\) |
|---------|---------------------------|-------------------------|---------------------------|------------|
| Bovine  | Brain                     | 0.33                    | 0.13\(^d\)                | 39         |
|         | Cerebellum                | 0.08                    | ND\(^d\)                  | ND         |
| Rat     | Brain                     | 0.23                    | 0.09                      | 39         |
|         | Synaptosomes              | 1.67                    | 0.58                      | 35         |

\(^a\) Crude postnuclear membranes were prepared from bovine brain cortices and cerebella and from rat brains; plasma membranes were purified from rat cerebrocortical synaptosomes (23).

\(^b\) To estimate the level of latrophilin expression, the membrane preparations were analyzed by Western blotting with an anti-latrophilin antibody as detailed under “Experimental Procedures.” For quantitation, immunostained bands were compared with serial dilutions of a known amount of purified latrophilin included on each blot.

\(^c\) Bovine and rat latrophilins were isolated by LTX affinity chromatography as described in the text; the yield of the pure protein was expressed in picomoles/mg of protein used for purification.

\(^d\) ND, not determined.
neuronal plasma membrane and then deliver the toxin to its physiologically active receptor and/or molecular target. As LTX interacts stronger with latrophilin than with neurexins (up to eight times, Fig. 2 and Ref. 22), it may gradually dissociate from neurexins and then bind to latrophilin. Rapid changes in the local Ca\(^{2+}\) concentration may facilitate this transfer. This hypothesis is consistent with the observation that not all LTX, bound to neuroblastoma cells in Ca\(^{2+}\)-free solutions, creates channels (30). These inactive toxin molecules are quickly converted to both receptors in Ca\(^{2+}\)-dependent receptor. The addition of Mg/EGTA in this case would release the toxin from neurexins and make it available to the alternative receptor.

Based on all evidence, we propose that latrophilin may participate in the LTX-triggered exocytosis either as the receptor or molecular target for the toxin. Future experiments will reveal the physiological function of latrophilin and its involvement in neurotransmitter release caused by \(\alpha\)-latrotoxin.

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