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Minireview

α-Latrotoxin receptor

Implications in nerve terminal function

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α-Latrotoxin is a potent stimulator of neurotransmitter release from nerve terminals. High affinity membrane α-latrotoxin receptor was purified in an active binding form. It is a membrane glycoprotein (M_r 160,000–220,000) which may be complexed to a smaller polypeptide (M_r 29,000). The structure of the receptor protein suggests that it may be a synapse-specific cell recognition molecule. Intracellularly, the α-latrotoxin receptor interacts with synaptotagmin, a calcium- and phospholipid-binding protein specifically localized in the synaptic vesicle membrane. This interaction may be important for targeting of synaptic vesicles to presynaptic release sites.

Neurotransmitter release; α-Latrotoxin; Neurexin; Synaptic vesicle; Synaptotagmin

1. INTRODUCTION

The major function of the nerve system is to perceive, process and output signals which control the functions of an organism. This is achieved by specific and very complex signaling between neuronal cells. To communicate, neurons form close contacts (called synapses) where low molecular weight neurotransmitters are released from the nerve terminal of a presynaptic neuron and received by the postsynaptic membrane of another cell. Neurotransmitters are accumulated and stored in specific cell organelles, synaptic vesicles. In the course of neuronal signal transduction the presynaptic membrane is depolarized and calcium channels are activated, which results in a calcium influx and subsequent exocytosis of synaptic vesicles. The physiology and pharmacology of neurotransmitter release was characterized in details but not until now have we started to understand what are the molecular mechanisms which underlie this phenomenon (for recent reviews see [1–5]).

One of the approaches which appears to be especially useful in the studies of different neural functions is the use of natural neurotoxins as tools to identify and characterize their target molecules. Several neurotoxins are known to influence nerve terminal function. Botulinum and tetanus toxins block neurosecretion by a selective proteolysis of a synaptic vesicle protein [6]. α-Latrotoxin is a well-characterized universal stimulator of synaptic vesicle exocytosis and neurotransmitter release [7] which has been extensively employed in physiological studies of neurosecretion. It has been particularly useful in establishing the vesicular theory of neurotransmitter release [8,9]. The molecular mechanism of the α-latrotoxin action is not clear yet. However, it was found to include binding initially to a receptor protein which is localized exclusively in the presynaptic membrane [10]. The scope of this review is to summarize the most recent findings of the α-latrotoxin studies focusing to the characteristics of the membrane-bound α-latrotoxin receptor.

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2. α-LATROTOXIN: THE MODE OF ACTION

α-Latrotoxin (M_r 116,000 Da) is one of the major protein components of black widow spider venom which is selectively toxic to vertebrates [11]. This toxin has been purified either by a combination of gel filtration and ion-exchange chromatography [11,12], or by immunoaffinity chromatography [13].

The physiological action of the α-latrotoxin is characterized by the following features:

1. Upon α-latrotoxin stimulation, the number of neurotransmitter quanta released correlates with the number of synaptic vesicles exocytosed, indicating that toxin-stimulated neurotransmitter release occurs via exocytosis [14].
2. Stimulation by toxin results in the exclusive release

of small synaptic vesicles whereas the neuropeptide containing large dense core vesicles are not involved [15].
3. α-Latrotoxin was found to be an effective secretion stimulator for all types of neurotransmitters (reviewed in [7]), thus its action should be targeted to the machinery, which is similar in the synapses of different types.
4. In comparison with physiological depolarization-driven release, α-latrotoxin stimulates the exocytosis of a much wider (probably all available) pool of synaptic vesicles [16]. Interestingly, under these conditions the proteins of synaptic vesicles get incorporated into the presynaptic membrane but do not intermix with its protein components [17,18].
5. Along with synaptic vesicle exocytosis, α-latrotoxin depolarizes the presynaptic membrane and causes an influx of Ca^{2+} ions, even in the presence of calcium channel antagonists, by induction of different channels [19]. Generally, the function of α-latrotoxin does not require extracellular Ca^{2+} provided that Mg^{2+} or another divalent cation is present [14,20]. It was also reported recently, that the effect of the toxin has both external Na^+-dependent and independent components [21].

In artificial membranes, α-latrotoxin can form cationic channels [22,23]. However, the properties of these channels are different from those induced in the presynaptic membrane [24]. A monoclonal anti-α-latrotoxin antibody which impairs the toxin stimulatory action but does not influence its binding to the receptor, also inhibits toxin-induced channels in artificial membranes [25]. Surprisingly, there is no homology of α-latrotoxin [26] with any known ion channel protein. However, a low-molecular weight protein which may be an ionophore, has been found in the toxin preparations [27].

α-Latrotoxin was also reported to induce the fusion of liposomes with the lipid bilayer when applied from the opposite side [28]. Thus, there is a possibility that after binding to its receptor, the toxin (or at least a part of the molecule) is translocated through the membrane, which results not only in channel formation, but also in the interaction with the synaptic vesicle membrane. It was also proposed that α-latrotoxin is translocated into the cytosol, and its domain containing twenty CDC10 (or ankyrin) repeats interacts with intracellular target [29].

3. IDENTIFICATION AND MOLECULAR CLONING OF THE α-LATROTOXIN RECEPTOR

The iodinated radioactive derivative of α-latrotoxin was used to identify high-affinity receptors in nerve tissues and also in differentiated PC12 cells [30–32]. The parallel studies of toxin binding and its stimulatory activity suggested that the function of α-latrotoxin requires binding to a specific cell surface receptor as an initial step [31]. Measuring toxin binding at different temperatures, two types of binding sites were detected in rat brain synaptosomes [33]. It was proposed that, depending on membrane fluidity, α-latrotoxin may complex with one or two receptor molecules [33].

An immunofluorescence study of the neuromuscular junction indicated that the α-latrotoxin receptor is localized exclusively in the presynaptic membrane [10]. This was also supported by a quantitative comparison of the receptor and synapsin distribution in brain [34]. Since the α-latrotoxin receptor appears to be a specific marker of the nerve terminal membrane, it is a very attractive target for further structural and functional studies.

An affinity chromatography approach was successfully utilized to purify the α-latrotoxin receptor [35–37]. Rat or bovine membranes were solubilized with Triton X-100 and the extract was applied to an agarose column containing immobilized α-latrotoxin. The eluted proteins exhibited a specific high-affinity toxin binding comparable to that of the membrane receptor (K_d close to 10^{-9} M) [35–37]. The receptor activity can be destroyed by proteases, denaturing agents, heating, and it is also partially inhibited by lectins [37], which agrees with the lectin antagonism to the toxin stimulatory activity [38]. However, the purified receptor as well as the solubilized one bind α-latrotoxin only in the presence of calcium [35,37], whereas the membrane receptor binds toxin even in the presence of EDTA [32,33,37]. After reconstitution in liposomes, the affinity-purified receptor does not require Ca^{2+} for toxin binding and is capable to form cation channels upon the addition of α-latrotoxin [39].

Currently, the α-latrotoxin receptor is thought to be composed of a protein which is a member of a family of highly homologous polypeptides ranging in size from 160,000 to 220,000 Da. These may be complexed with a smaller protein of M_r 29,000 Da. This conclusion is supported by the following lines of evidence:
1. Electrophoretic analysis detects 200K, 160K and 29K polypeptides as the major protein components in the affinity-purified receptor preparations [36,37,40,41].
2. In a sucrose density sedimentation experiment, the distribution of 160K and 200K proteins correlates strongly with the profile of the specific α-latrotoxin-binding activity. The sedimentation pattern of the 29K protein suggests that it may form a complex with either the 160K or the 200K protein and that its presence is not absolutely necessary for the toxin binding. When a mixture of toxin and receptor is sedimented, all three polypeptides as well as the toxin shift to a higher molecular mass, indicating the formation of stable complexes [40].
3. The 160K and 200K proteins can be separated by anion-exchange chromatography. After separation, both of these retain their high affinity α-latrotoxin binding [37] and can complex with the 29K protein [40].
4. An antibody, which stains both the 200K and 160K proteins on Western blots, also immunoprecipitates the

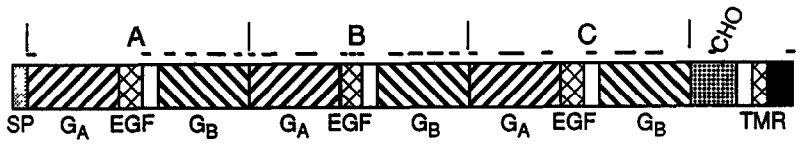

Fig. 1. Domain structure of the α-latrotoxin receptor (deduced from the neurexin Iα cDNA). The protein is shown as bar diagram with amino terminus on the left. The matching peptide sequences derived from the affinity-purified receptor are outlined as solid bars. The signal peptide (SP) is followed by three repeats (A, B and C), each containing a central EGF-domain. Left and right arms (Ga and Gb) of each repeat are homologous to the laminin A G-domain repeats. The single transmembrane segment (TMR) of the receptor is preceded by putative O-linked sugar-binding domain (CHO). Modified from [29,42].

α-latrotoxin-binding activity from total rat brain extracts [42].

Purified 160K and 200K proteins give indistinguishable peptide maps and show strong immune cross-reactivity, but they have a different carbohydrate composition [37]. However, treatment with different glycosidases does not allow to reduce them to electrophoretic bands of the same size [42]. The 29K protein is not related to the 160K and 200K proteins since an antibody against the 29K protein does not recognize any of the larger receptor components [40].

The information on internal peptide sequences obtained from a mixture of 160–220K proteins was used to clone cDNAs representing a novel family of highly polymorphic cell surface membrane proteins which were named neurexins [42]. Neurexin mRNAs were only found in brain-related tissues [42], which perfectly correlates with the distribution of the α-latrotoxin receptor [7]. At least three different neurexins exist [29]. Each of these have two forms (α, the longer; and β, the shorter, sharing the same C-terminal region), which are probably the results of transcription from alternative promoters. In addition, all the neurexin cDNAs are alternatively spliced at least at 5 internal positions [42].

The α-latrotoxin receptor is probably a splice variant of neurexin Iα. More than 20% of the neurexin Iα sequence is identical with the amino acid sequences of randomly distributed peptides derived from the purified receptor (Fig. 1). An antibody against a cytoplasmic C-terminal fragment of neurexin Iα immunoprecipitates the α-latrotoxin-binding activity [42]. However, attempts to express the functionally active receptor in COS cells have not so far been successful [42]. An explanation might be that the expression of α-latrotoxin-binding activity requires a specific combination of splicing at different sites of the neurexin Iα mRNA. Alternatively, there is a possibility that during the expression in COS cells the protein does not undergo some specific posttranslational modification available only in neurons. Since oocytes injected with the total brain mRNA acquire the ability to respond to α-latrotoxin [43], this latter approach might become useful to identify components necessary for proper α-latrotoxin receptor expression.

4. POSSIBLE FUNCTIONS OF THE α-LATROTOXIN RECEPTOR

The α-latrotoxin receptor and, generally, neurexins are nerve-specific proteins, which are localized in presynaptic membranes [10,34,26,42]. What might be the physiological function of these proteins in the nerve terminal? α-Latrotoxin receptor is a membrane protein with only one transmembrane segment, a very short C-terminal cytoplasmic tail and an extensive N-terminal extracellular domain which is glycosylated and also contains three EGF-like repeats characteristic for extracellular proteins (Fig. 1). These structural features make it unlikely that the receptor acts as an ion channel. There are six domains in the extracellular part of the receptor homologous to the laminin A G-domain repeats, which are also found in agrin, perlecan, and slit [42]. These proteins are thought to be important in axon guidance and synaptogenesis. Thus, the extracellular part of the α-latrotoxin receptor was proposed to perform a cell adhesion function in the nerve terminal [26,42]. The identification of cell structures interacting with the neurexin extracellular domain would be of help to verify this hypothesis.

It seems likely that the function of the short cytoplasmic tail of the neurexins is to complex with synaptic vesicles (Fig. 2) via the cytoplasmic domain of synaptotagmin [41,44]. Synaptotagmin is a membrane-bound calcium- and lipid-binding glycoprotein, specifically localized in synaptic vesicles (reviewed in [3]). Synaptotagmin is implicated in neurotransmitter release [45] and was proposed to act as a calcium sensor on the synaptic vesicle membrane [46].

Synaptotagmin co-purifies with the solubilized α-latrotoxin receptor and this interaction is so specific that synaptotagmin can be purified in one step by affinity chromatography of the synaptic vesicles detergent extract on a column with immobilized α-latrotoxin receptor [41]. Synaptotagmin binds to cytoplasmic domains of different neurexins, which appear to be highly conserved. A study with deletion mutants allowed to localize the synaptotagmin-binding site to the 40 amino acid residues of the C-terminus of neurexins [44].

Synaptotagmin was also reported to co-immunopre-

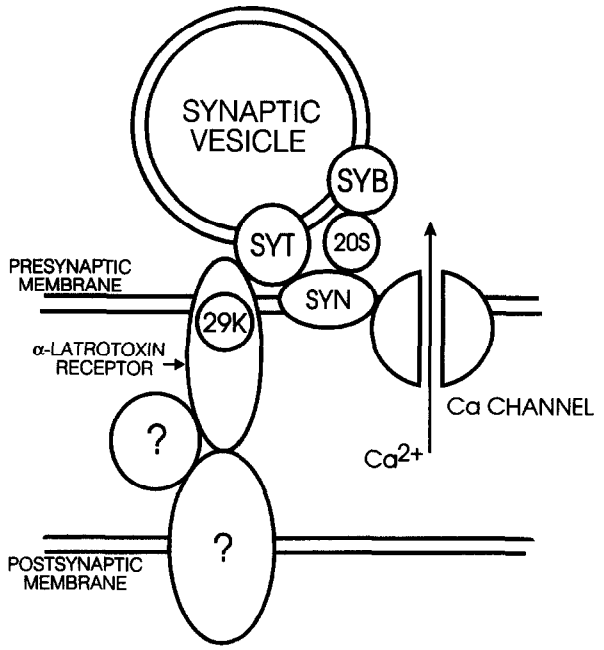

Fig. 2. Schematic model of the proposed organization of the α-latrotoxin receptor and other proteins at the nerve terminal. Based on the identified interactions of the α-latrotoxin receptor with synaptotagmin (SYT) [41] and 29K protein [40], synaptotagmin with syntaxin (SYN) and ω-conotoxin receptor (Ca CHANNEL) [47,48], and syntaxin with 20 S fusion particle (20 S) and synaptobrevin (SYB) [49], which is a target for tetanus and botulinum neurotoxins [6]. In vivo, the outlined interactions should not necessarily occur simultaneously and may be controlled by levels of calcium, ATP, GTP, etc.

cipitate with the ω-conotoxin receptor [47,48], which is a presynaptic calcium channel protein, and with syntaxin [48], another presynaptic membrane protein implicated in membrane fusion [49]. Thus, the interactions involving the α-latrotoxin receptor, or any other neurexins, may be important for the docking of synaptic vesicles at the release sites with proper positioning in relation to calcium channels, which induce neurotransmitter release (Fig. 2).

Synaptotagmin is phosphorylated by casein kinase II in vivo [50]. The calcium-dependent phosphorylation of synaptotagmin is specifically inhibited by the α-latrotoxin receptor [41]. Thus, it is possible that this phosphorylation event is important for the regulation of a number of synaptic vesicles available for exocytosis as a function of amount of entered calcium. Such type of regulation may be involved in long-term potentiation events.

5. CONCLUSIONS

The use of α-latrotoxin as a tool has allowed to obtain important evidence supporting the vesicular model of neurotransmitter release. The currently available data suggest that there are two steps in toxin functioning. The first step is the binding to the membrane-bound receptor, and the second one may involve the formation of a cation channel and/or the interaction with intracellular components, as a result of the translocation of α-latrotoxin across the cytoplasmic membrane. The structure of the α-latrotoxin receptor indicates that it may be a membrane protein important for cell recognition in the synapse. It probably acts as a toxin acceptor and is not involved in transmembrane signalling. The intracellular targets of the toxin are yet to be identified.

Undoubtedly, future studies will develop in both directions: to a further investigation of the mechanism of the α-latrotoxin function, which is important for understanding neurotransmitter release, and also to a study of the physiological function of the α-latrotoxin receptor and associated proteins.

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