

# $\alpha$ -Latrotoxin and Its Receptors

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**Abstract**  $\alpha$ -Latrotoxin ( $\alpha$ -LTX) from black widow spider venom induces exhaustive release of neurotransmitters from vertebrate nerve terminals and endocrine cells. This 130-kDa protein has been employed for many years as a molecular tool to study exocytosis. However, its action is complex: in neurons,  $\alpha$ -LTX induces massive secretion both in the presence of extracellular Ca<sup>2+</sup> (Ca<sup>2+</sup><sub>e</sub>) and in its absence; in endocrine cells, it usually requires Ca<sup>2+</sup><sub>e</sub>. To use this toxin for further dissection of secretory mechanisms, one needs an in-depth understanding of its

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functions. One such function that explains some  $\alpha$ -LTX effects is its ability to form cation-permeable channels in artificial lipid bilayers. The mechanism of  $\alpha$ -LTX pore formation, revealed by cryo-electron microscopy, involves toxin assembly into homotetrameric complexes which harbor a central channel and can insert into lipid membranes. However, in biological membranes,  $\alpha$ -LTX cannot exert its actions without binding to specific receptors of the plasma membrane. Three proteins with distinct structures have been found to bind  $\alpha$ -LTX: neurexin I $\alpha$ , latrophilin 1, and receptor-like protein tyrosine phosphatase  $\sigma$ . Upon binding a receptor,  $\alpha$ -LTX forms channels permeable to cations and small molecules; the toxin may also activate the receptor. To distinguish between the pore- and receptor-mediated effects, and to study structure-function relationships in the toxin,  $\alpha$ -LTX mutants have been used. At least one non-pore-forming  $\alpha$ -LTX mutant can activate latrophilin, a G protein-coupled receptor, causing release of  $\text{Ca}^{2+}$  from intracellular stores. Latrophilin action still requires  $\text{Ca}^{2+}_e$  and may trigger transmitter secretion either by itself or by activating  $\text{Ca}^{2+}$ -channels and/or inducing  $\text{Ca}^{2+}$  waves. These results reveal two  $\text{Ca}^{2+}_e$ -dependent mechanisms of  $\alpha$ -LTX action (membrane pore formation and signalling via latrophilin), but how  $\alpha$ -LTX triggers  $\text{Ca}^{2+}_e$ -independent neurotransmitter release still remains unexplained. Hypotheses for this action include direct interaction with intracellular components involved in exocytosis or the effects of  $\alpha$ -LTX pores.

## 1 $\alpha$ -LTX and Release of Neurotransmitters

The notorious black widow spider (*Latrodectus* genus) and its venom, first studied by ancient Greeks (Aristotle 350 B.C.), entered the era of modern science in the 1930's with the discovery of its proteinaceous active principles (D'Amour et al. 1936) and, following the demonstration that it affects synaptic neurotransmitter release (Longenecker et al. 1970) and the isolation of toxic components (Frontali et al. 1976), gripped the attention of neurobiologists. It played a role in the debate leading to the  $\text{Ca}^{2+}$  (Augustine et al. 1987) and vesicular quantum (Ceccarelli and Hurlbut 1980) paradigms of neurotransmission. However, some details of  $\alpha$ -LTX actions still remain mysterious, despite its widespread use as a stimulant of neurosecretion.

The venom contains at least 86 unique proteins (Duan et al. 2006), including several homologous LTXs which play a role in its toxicity to insects and crustaceans (Grishin 1998), with only one,  $\alpha$ -LTX, targeting vertebrates specifically; reviewed by Rosenthal and Meldolesi (1989).  $\alpha$ -LTX is usually isolated from spider venom by conventional chromatography (Frontali et al. 1976; Tzeng et al. 1978), but to achieve homogeneity and remove contaminants (Volkova et al. 1995; Pescatori et al. 1995) that may endow the preparation with uncharacteristic properties (Umbach et al. 1998), preparative native electrophoresis should ideally be used (Ashton et al. 2000).