BIOPHYSICS AND BIOCHEMISTRY

Activation of α-Latrotoxin Receptors in Neuromuscular Synapses Leads to a Prolonged Splash Acetylcholine Release

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The mechanisms of acetylcholine release in presynaptic terminals of motoneurons induced by mutant α-latrotoxin (LT^{N4C}) were analyzed. In contrast to wild-type α-latrotoxin that causes both continuous and splash secretion of acetylcholine and necessarity block neuromuscular transmission, LT^{N4C} causes only splash release lasting over many hours. Thus, activation of α-latrotoxin receptors controls long-lasting enhanced secretion of acetylcholine.

Key Words: *neuromuscular transmission; acetylcholine release; latrophilin; latrotoxin*

Acetylcholine release from the presynaptic motoneuron terminals causes generation of action potential in the myofibril membrane and thus leads to muscle contraction. It is well known that the maximum frequency of muscle contraction and the effort produced depends critically on myofibril metabolism, which, in turn, can be appreciably accelerated and adapted as a result of training. However, the work of even highly trained muscles depends on the level of innervation, degree of motoneuron excitability, frequency and synchronism of their activation, and safe work of the neuromuscular terminals. We studied these neuronal aspects of the neuromuscular activity and the possibility of their artificial regulation.

One of the most potent stimulants of neurotransmitter release, particularly of acetylcholine release in the neuromuscular terminals, is neurotoxin from venom of caracurt spider *Latrodectus lugubris* (α-latrotoxin, αLT). This toxin is characterized by strictly presynaptic effect [5] and is often used as an instrument for studies of the synaptic transmission mechanisms [6]. Since αLT exhibits its activity through protein receptors, the nature and signal functions of these receptors have attracted special interest [5-7]. On the other hand, αLT tetramers can form cation-permeable pores in the cell membrane [1,4]. These pores are particularly selective for calcium ions, which directly stimulate exocytosis of synaptic vesicles in nerve terminals. Because of this, the mode of αLT action remained so obscure for a long time, that some scientists even doubted that αLT receptors were capable of modifying the neurotransmitter secretion [3].

However, the situation changed after creation of mutant αLT (LT^{N4C}) [3,9]. This recombinant protein differs from the wild type toxin by inability to form tetramers and hence, pores in the cell membrane [2,9]. On the other hand, LT^{N4C} can bind αLT receptors and, which is very important, induce the release of at least some neurotransmitters. Due to these characteristics, LT^{N4C} is an interesting tool for evaluation of the role of presynaptic receptors and potential model for the creation of synthetic stimulants of the neuromuscular transmission.

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We studied acetylcholine secretion caused by LT^{N4C} and evaluated whether it can be used for long-lasting stimulation of nerve terminals.

MATERIALS AND METHODS

Experiments were carried out on neuromuscular preparations (flexor digitorum brevis) of the mouse hind paws. The preparations were fixed with entomological pins in Petri dishes precoated with Sylgard silicone polymer (Dow Corning) and washed in saline containing 2 mM calcium and 1 μM tetrodotoxin (Latoxan) at permanent oxygenation. Spontaneous presynaptic activity was evaluated by postsynaptic registration of miniature end-plate potentials (MEPP). Acute glass microelectrodes with tip diameter <0.5 μ and 30-60 MΩ resistance filled with 5 M ammonium acetate were used for this purpose. The electrodes were inserted in myocyte cytoplasm using a PatchStar motorized micromanipulator (Scientifica), the procedure was controlled under an SMZ45 binocular microscope (Nikon). The membrane potentials were recorded using a system consisting of an Axoclamp 2B pre-amplifier (Axon Instruments), LPF202A amplifier with a high-frequency filter (Warner Instruments), Hum Bug harmonic frequency quencher (Quest Scientific), Digidata 1322A digital transformer (Axon Instruments), and microcomputer with AxoScope 10 software (Axon Instruments).

Stimulation was carried out by adding αLT or LT^{N4C} to concentrations of 0.1-2.0 nM to the bathing solution. α-Latrotoxin was isolated from the venom of caracurt spider *Latrodectus lugubris* (Fauna Laboratories Ltd.) as described previously [1]. Recombinant LT^{N4C} was purified from medium conditioned by Hi5 cells infected with baculovirus carrying mutated toxin gene [9,10].

The data were analyzed using Mini Analysis 6 software (Synaptosoft).

RESULTS

The frequency of MEPP at rest varied from cell to cell, but never exceeded 1 Hz. After addition of αLT or LT^{N4C}, the frequency of miniature potentials always increased, but only after a certain delay, which was comparable for both toxins (10 min on average). However, the effects caused by α-LT and LT^{N4C} principally differed (Fig. 1).

The wild-type toxin initially induced splashes of neurotransmitter secretion (Fig. 1, *a*). These splashes depended on the presence of calcium ions and corresponded to the release of 250±30 individual vesicles at the mean frequency of about 60 Hz. This clonic (splash) exocytosis was then replaced by tonic (permanent) release of acetylcholine, which attained 100-150 Hz and then gradually decreased. Tonic activity did not depend on calcium ions, always led to complete cessation of secretion, and usually lasted no longer than 30 min.

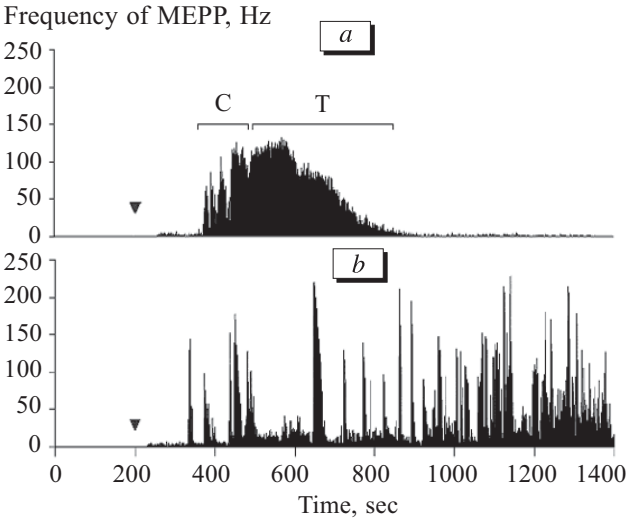

Fig. 1. Effect of 1 nM solutions of wild type αLT (*a*) and mutant LT^{N4C} (*b*) on the frequency of spontaneous MEPP. Arrows show the time of toxin addition. C: clonic exocytosis; T: tonic release of acetylcholine.

LT^{N4C} also caused clonic secretion of acetylcholine (Fig. 1, *b*). However, in contrast to wild type toxin, mutant toxin never led to tonic exocytosis and acted during the entire period of recording. The maximum frequency of vesicle release reached 220 Hz, mean frequency being 63±5 Hz. The mean number of vesicles released during each splash was 247±35. This clonic activity lasted for several hours without appreciable changes in the frequency of splashes or frequency of MEPP in each splash (maximum period of recording from one myocyte was longer than 5 h).

Hence, both toxins exhibited similar parameters of activation of clonic release of vesicles except for tonic exocytosis. The mean number of individual vesicles released during each splash was 250. The mean duration of active exocytosis splash was 4 sec and a period of 20 sec.

It is known that wild type αLT (but not mutant LT^{N4C}) forms membrane pores [1,9]. Moreover, the part of αLT activity related to the formation of membrane pores does not depend on calcium ions [8]. Both toxins well react with the receptors on the presynaptic membrane. Hence, tonic secretion in experiments with αLT is due to pores formed by this toxin in the presynaptic membrane, while clonic secretion in experiments with both toxins seems to be mediated by their interactions with the same class of presynaptic receptors. The fact that αLT failed to cause lasting clonic exocytosis, similar to that induced by LT^{N4C}, is explained by its destructive effect on nerve terminals of the pores formed by the normal toxin.

An important conclusion from this study is the hypothesis according to which clonic (splash) release of neurotransmitters is regulated through activation of presynaptic receptors. The nature of these receptors is now investigated, though, based on the above characteristics of secretion and the known intracellular mechanisms related with latrophilin 1 [2,7], we can say with a high degree of probability that splash release of acetylcholine is caused by latrophylin activation.

Based on the lactrophilin-mediated activity characteristics, we suggest that the toxin complex with this receptor hyperstimulates the important intracellular cascade in the presynaptic terminals, which leads to active secretion of acetylcholine. This mechanism can be used for external control and regulation of neuromuscular activity.

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