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α-Latrotoxin-stimulated GABA release can occur in Ca^{2+}-free, Na^{+}-free medium

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Abstract

We have studied [^{14}C]GABA release from synaptosomes induced by native and monoclonal antibodies-modified α-latrotoxin (LTX). Modification of LTX eliminates the toxin's ability to increase [Ca^{2+}]$_i$ influx into synaptosomes. It has been shown that native LTX does not change ^{22}Na influx into rat brain synaptosomes. Both toxin forms studied, native and modified by monoclonal antibodies, stimulate [^{14}C]GABA release from synaptosomes in divalent-free medium where sodium was substituted by equimolar concentrations of choline chloride. Native toxin induces a more rapid stimulation of [^{14}C]GABA release than the modified one. It was suggested that the difference in the mediator release rates is not accounted for by the inability of modified toxin to form active ion channels in synaptosomal plasmalemma, but most probably by the state of toxin–receptor complexes.

Key words: α-Latrotoxin; Monoclonal antibody A4; Rat brain synaptosome; GABA release; ^{22}Na influx

1. Introduction

It is well known that α-latrotoxin (LTX) – the major toxic component of black widow spider venom – can induce neurotransmitter release from nerve terminals and PC cells both in Ca^{2+}-supplemented and Ca^{2+}-free medium. This ability of LTX is accounted for by toxin-induced sodium influx into nerve terminals and as a consequence by the change of sodium electrochemical gradient that ensures the reverse of the amino acid carriers. In addition it was suggested that in Ca^{2+}-free medium non-vesicular neurotransmitter amino acids are released through the large pores formed by LTX–receptor complexes [1,2].

As recently was established, the modulation of LTX by monoclonal antibodies A4 (mAbA4) creates the toxin form that in contrast to native toxin does not increase ^{45}Ca influx in synaptosomes, but retains the secretogenic ability [3]. This modified toxin form may be a valuable tool for investigation of the mechanism of toxin action, particularly the role of ionic gradients in release process stimulation. In present study we have compared the secretogenic effect of native LTX and LTX modified by mAbA4, and the dependence of this effect on sodium ions.

2. Experimental

2.1. Purification of α-latrotoxin

LTX was isolated from *Latrodectus mactans tredecimguttatus* venom gland by mAb immunoaffinity chromatography [4].

2.2. Modification of α-latrotoxin with monoclonal antibodies A4

Anti-α-latrotoxin mAbs used in these experiments have been previously described in detail [3]. For of LTX-modification with mAbs, a stock solution of LTX (5×10^{-7} M) was incubated with mAbsA4 (2×10^{-6} M) for 1 h at 20°C. The effect of LTX modified by mAbs was referred to a control obtained by adding the same amount of appropriate immunoglobulin fraction to the toxin.

2.3. Preparation of synaptosomes

Synaptosomes were purified from cortical homogenates of rats (100–200 g, males) by differential and density-gradient centrifugation as described by Cotman [5] with modification described in [3].

2.4. GABA release from synaptosomes

The loading of synaptosomes (2 mg of protein/ml) with [^{14}C]GABA was performed for 5 min at 30°C in standard salt solution of the following composition (in mM): 126 NaCl, 5 KCl, 1.4 $MgCl_2$, 1 $CaCl_2$, 1 Na_2HPO_4, 20 HEPES, pH 7.4, and 10 glucose in the presence of 10 mM aminooxyacetate. [^{14}C]GABA was added to a concentration 5×10^{-7} M (0.1 mCi/ml; sp.act. 232 mCi/mmol; Amersham). The loading was stopped by cooling and ten-fold dilution of samples in ice-cold media of appropriate composition without [^{14}C]GABA. The synaptosomal suspension was then precipitated by centrifugation at $5{,}000 \times g$ (4°C) and resuspended up to 1 mg protein per ml in one of the following media: (i) standard salt solution (SSS); (ii) calcium-free solution – SSS devoid of calcium and with addition of 0.1 mM EGTA; (iii) sodium-free solution – SSS where sodium ions are substituted by an equimolar concentration of choline chloride; (iv) calcium-free and sodium-free solution – solution (iii) devoid of calcium and with 0.1 mM EGTA. The solutions used for [^{14}C]GABA efflux experiments also contained 10 mM aminooxyacetate. Measurement of GABA release was carried out as described [3].

2.5. Sodium influx

Freshly prepared synaptosomes were resuspended at 4 mg of protein per ml in SSS. ^{22}Na (2 μCi/ml) was added after 15 min pre-incubation at room temperature to ensure a return to steady-state conditions. LTX (final concentration 15 nM) was added to the synaptosomal suspension simultaneously with ^{22}Na. To terminate ^{22}Na uptake at a definite time, 0.5 ml aliquots were removed and filtered through Whatman GF/C filters. The filters were washed three times with 4 ml unlabeled washing solution (126 mM NaCl, 5 mM KCl, 20 mM HEPES, pH 7.4). The radioactivity was determined by scintillation counting in Delta 300 counter (USA).

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2.6. $[Ca^{2+}]_i$

Synaptosomes were loaded with fura-2 and $[Ca^{2+}]_i$ was measured as described [6]. All solutions used in experiments with fura-2 contained 2.5 mM probenecid.

3. Results and discussion

In [^{14}C]GABA release experiments both native LTX and LTX modified by mAbsA4 have been used. As was recently shown, the interaction of LTX with mAbsA4 completely eliminates only one effect of LTX – the toxin's ability to increase the ^{45}Ca influx into synaptosomes [3]. To determine at what level of synaptosomal $[Ca^{2+}]_i$ mAbsA4-modified LTX induces GABA release, the experiments with synaptosomes loaded with fura-2 were performed. It was established that mAbsA4-modified LTX, in contrast to native toxin, does not induce an increase of synaptosomal calcium concentration (Fig. 1). This result is in accordance with facts established by monitoring of ^{45}Ca influx [3] and in addition shows that modified LTX is incapable of mobilizing calcium from intracellular stores. Thus evidence was obtained that under the interaction of mAbsA4-modified toxin with membrane receptor, changes to both synaptosomal calcium concentration and calcium gradient on synaptosomal plasmalemma do not occur and it may be concluded that no ion channels were formed when the toxin structure was modified by this type of monoclonal antibody.

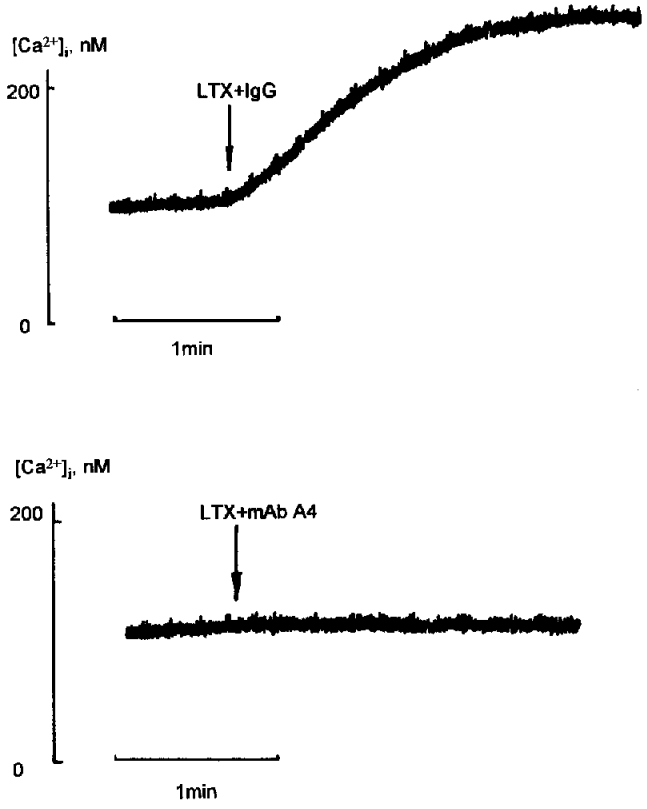

Fig. 1. The influence of native LTX and mAbA4-modified LTX on synaptosomal $[Ca^{2+}]_i$. Fura-2-loaded synaptosomes (0.1 mg protein/ml) were incubated in standard salt solution at 37°C for 7 min to gain steady-state conditions. After that LTX or LTX-mAbA4 complex was added to final concentration of 5×10^{-9} M.

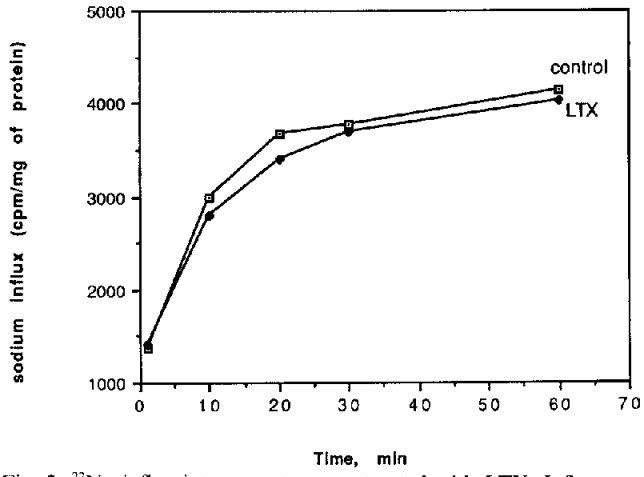

Fig. 2. ^{22}Na influx into synaptosomes treated with LTX. Influx was measured at room temperature as described in section 2. LTX was added to a final concentration of 1.5×10^{-8} M simultaneously with ^{22}Na.

The next question investigated in this study is what changes in sodium permeability of presynaptic membrane are induced by LTX. Little is known about the effect of LTX on synaptosomal sodium permeability. Such investigations were carried out on PC cells by the patch-clamp method [7]. Results obtained in this study show that LTX induces not only increase in Ca^{2+} permeability, but also that of Na^+ and K^+. In the present study, the toxin-induced sodium influx into rat brain synaptosomes was monitored with ^{22}Na both in Ca^{2+}-supplemented and Ca^{2+}-free medium with 0.1 mM EGTA. As illustrated in Fig. 2 even in the presence of high LTX concentration (15 nM) the ^{22}Na flow rate in Ca^{2+} medium does not exceed the rate in the absence of LTX (control rate). The same results were obtained in Ca^{2+}-free medium with EGTA (data not shown). It is noteworthy that such a high concentration of toxin induces a rapid and sharp increase in ^{45}Ca influx into synaptosomes. Immutability of synaptosomal sodium permeability under LTX action is in accordance with our earlier data concerning the inability of LTX to depolarize the synaptosomal plasmalemma in the absence of bivalent ions in the medium [8]. If that is so, the channel formed by LTX in synaptosomes may be suggested to be impermeable for sodium ions.

[^{14}C]GABA release from rat brain synaptosomes was determined in standard salt medium, in the same medium where Ca^{2+} is omitted and EGTA added, in Na^+-free medium and in Ca^{2+}-free, Na^+-free medium. In Fig. 3 it can be seen that mAbA4-modified toxin can induce [^{14}C]GABA release from preliminary loaded synap-

tosomes and the quantity of released neurotransmitter over 10 min is the same in all experimental media studied. It was shown that the rate of control release and the rate of [^{14}C]GABA release induced by the mAbA4-modified toxin are linear for the first 15 min [3]. It is noteworthy that the absence of Ca^{2+} or Na^{+} in different media does not change the control release. So results of these experiments suggest that the ability of mAbA4-modified toxin to induce the neurotransmitter release is independent of external Ca^{2+} and Na^{+}.

The results of [^{14}C]GABA release experiments under the action of native and mAbA4-modified LTX in calcium-free, sodium-free medium are represented in Fig. 4. It can be seen that curves of release in these two cases are different. Earlier this distinction was shown in complete salt medium [3]. Native toxin stimulates rapid release for the first 5 min and then the rate of release begins to decline. Under the interaction of modified toxin with synaptosomes the lag period can be observed, and then [^{14}C]GABA release occurs at a low and constant rate. The same difference in mode of action of these two toxin forms was found in all conditions studied (see section 2). Thus, it was realized that the secretogenic effect under the influence of mAbA4-modified LTX can occur without ionic channel formation. We therefore suggest that in the case the neurotransmitter release it is due to exocytosis, but not to the non-specific leakage from the cytoplasmic pool. In addition we want to emphasize that under the described conditions, the exocytotic process can take place even when both the bivalent cations, Ca^{2+} and Mg^{2+}, were omitted from the sodium-free medium (data not shown). It is necessary to bear in mind that the

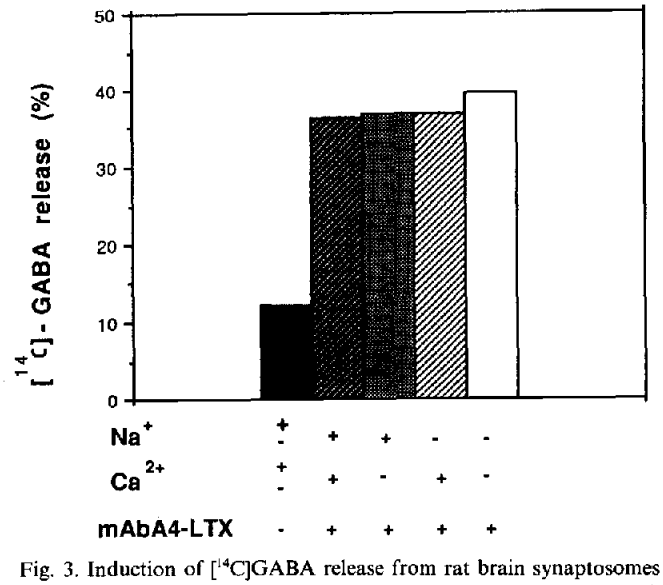

Fig. 3. Induction of [^{14}C]GABA release from rat brain synaptosomes by the mAbA4-LTX complex. The suspension preloaded with [^{14}C]GABA right before the efflux experiments was incubated for 5 min at 30°C, then the initial level of [^{14}C]GABA was measured. That moment was taken as point zero. Time of release was 10 min. Final concentration of LTX was 5×10^{-9} M. LTX was added at zero point.

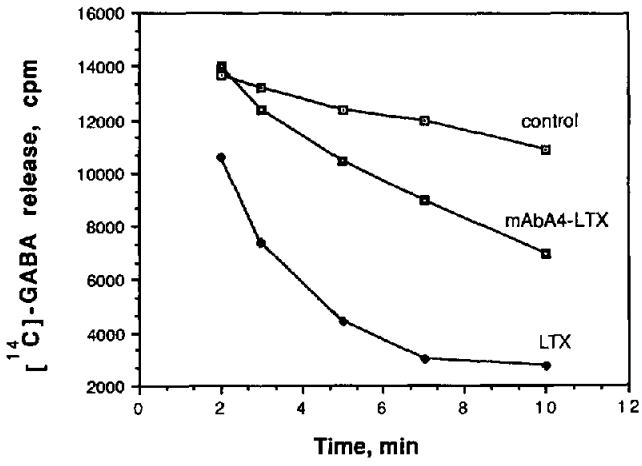

Fig. 4. Stimulation of [^{14}C]GABA release from synaptosomes by native LTX and mAbA4–LTX complex in calcium-free, sodium-free medium. The experimental conditions are similar to those indicated in Fig. 3.

realization of the toxic action of LTX in the bivalent cation-free media is possible only in ionic medium. In ion-free sucrose medium LTX does not exert its toxic action [9]. These data do not conform with the opinion that Ca^{2+} or Mg^{2+} is necessary for the toxin action in the absence of Na^{+} [1].

The results obtained allow us to affirm that realization of LTX stimulation of mediator release does not occur by changes in ion gradients. It is worth pointing out that some other investigators have not recognized sodium as an obligatory factor for induction by LTX neurotransmitter release [10]. However, McMahon et al. [2] observed that toxin-induced release of endogenous cytoplasmic glutamate from synaptosomes was decreased in Na^{+}-free medium. As can be judged by our experimental data, LTX does not induce an increase in sodium synaptosomal permeability and therefore does not influence the operation of the amino acid carrier.

The results of our experiments permit the conclusion that for the realization of presynaptic action of LTX, changes in sodium and calcium plasma membrane permeability are not essential. More probable is that the interaction of LTX with the membrane receptor induces the formation of a multimeric protein complex that enables the triggering of the neurosecretory process [11].

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