Fusion of negatively charged phospholipid vesicles by α-latrotoxin

V.K. Lishko, Ya.T. Terletskaya and I.O. Trikash

Department of Neurochemistry, A.V. Palladin Institute of Biochemistry, Academy of Sciences, Ukrainian SSR, Kiev 252030, USSR

Received 23 April 1990

α-Latrotoxin-induced fusion of liposomes has been described using large unilamellar vesicles composed of phosphatidylcholine/phosphatidylethanolamine/cardiolipin at a molar ratio of 2:3:5. Vesicle fusion was monitored by terbium/dipicolinic acid assay as well as by fluorescence energy transfer measurement. The enhancement of the fusogenic effect of LTX by low concentrations (0.1–3 mM) of $CaCl_2$ has been demonstrated. The efficiency of other divalent cations on the LTX fusogenic activity was shown to decrease in the sequence Ca > Cd > Sr > Mg > Ba. LTX-induced fusion was accompanied by the increase of vesicle size measured by laser correlation spectroscopy. It is concluded that fusogenic action of LTX may be involved in its effect on synaptic apparatus.

α-Latrotoxin; Membrane fusion; Liposome

1. INTRODUCTION

Membrane fusion is involved in a number of cellular functions. It is well established that the secretory process of neurotransmitters in the nervous system occurs as a result of fusion of synaptic vesicles with presynaptic membrane. This exocytotic release of neurotransmitter is triggered by free Ca^{2+}.

LTX is a presynaptic toxin isolated from the venom of black widow spider (*Latrodectus mactans tredecimguttatus*). It is believed that the mode of its action is based on its possibility to form Ca^{2+}-selective ionic channels in presynaptic membranes [1]. However, it has been shown that the presynaptic effect of LTX occurs also in the absence of calcium ions in the extracellular medium [2]. We supposed that LTX by itself could trigger the fusion process. The experiments with the model liposome-planar lipid membrane system [3] and with cell-free model of neurosecretion consisting of synaptic vesicles and synaptic plasma membrane [4] substantiates this assumption.

In this paper, we have directly demonstrated the fusogenic activity of LTX in liposome experiments.

Correspondence address: Ya.T. Terletskaya or I.O. Trikash, Department of Neurochemistry, A.V. Palladin Institute of Biochemistry, Ukrainian Academy of Sciences, 9 Leontovitch Street, 252030 Kiev, USSR

Abbreviations: LTX, α-latrotoxin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; CL, cardiolipin; Chl, chlorophyll; DPA, dipicolinic acid; EDTA, ethylenediaminetetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

2. MATERIALS AND METHODS

2.1. *Materials*

PC, PE and CL were obtained from the Laboratory of Biotechnology (TINRO, Vladivostok, USSR). All phospholipids were assayed for purity on thin-layer chromatography and proved to be pure. Phospholipid was determined by phosphate assay [5]. $TbCL_3 \cdot 6H_2O$ was obtained from Chimreactiv (Kiev, USSR). Dipicolinic acid (pyridine-2,6-dicarboxylic acid) was from Sigma Chemical Co. Chlorophylls *a* and *b* were purchased from the Institute of Bioorganic Chemistry in Kiev, Hepes from Serva. LTX was purified by means of FPLC (Pharmacia) with a Mono-Q column [6].

2.2. *Liposome preparation*

Large unilamellar vesicles consisting of a ternary mixture of PC/PE/CL in the molar ratio 2:3:5, respectively, were prepared by reverse phase evaporation method [7]. The average size of liposomes was determined by the method of laser correlation spectroscopy [8].

2.3. *Fusion assay*

The Tb-dipicolinic acid fusion assay [9] was used for fusion monitoring. All measurements were carried out on a Hitachi 650-IOS fluorescence spectrophotometer at excitation 276 nm and emission 545 nm through >530 nm cutoff filter. Assays for the fusion of vesicle content were carried out in the medium of 100 mM NaCl, 5 mM Hepes, 0.1 mM EDTA, pH 7.4, and Tb- plus DPA-vesicles (50 μM lipid each).

Fusion experiments were conducted using resonance energy transfer method with chlorophylls *a* and *b* [10]. Fluorescence was measured at excitation wavelength of 465 nm, emission - 660 nm for Chl *b*, and 672 nm for Chl *a* through >640 cutoff filter.

3. RESULTS AND DISCUSSION

Fig. 1 shows the typical experiment demonstrating the effect of LTX on vesicle fusion. Two populations of vesicles (one containing Tb, the other - dipicolinic acid) were mixed and LTX was added to the vesicle suspension. Clearly, the extent of fusion was dependent on the

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

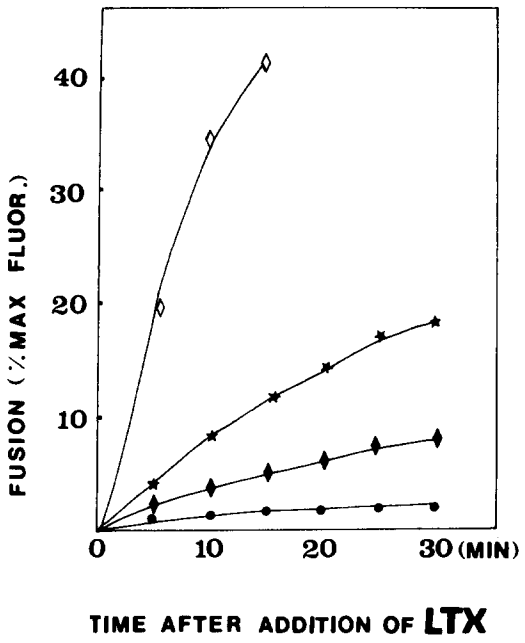

Fig.1. Kinetics of fusion liposomes as a function of α-latrotoxin concentrations. (●) 10^{-8} M; (♦) 5×10^{-8} M; (★) 10^{-7} M; (◇) 5×10^{-7} M. The maximum fluorescence (at 100% fusion) was set by lysing of Tb-containing vesicles with 0.5% sodium cholate in the presence of dipicolinic acid without EDTA.

LTX concentration. The rate of membrane fusion under the low LTX concentration (10^{-8} M) was very small, but it increased dramatically at 5×10^{-7} M. Little wonder that LTX promoted the fusion of other negatively charged phospholipid vesicles containing

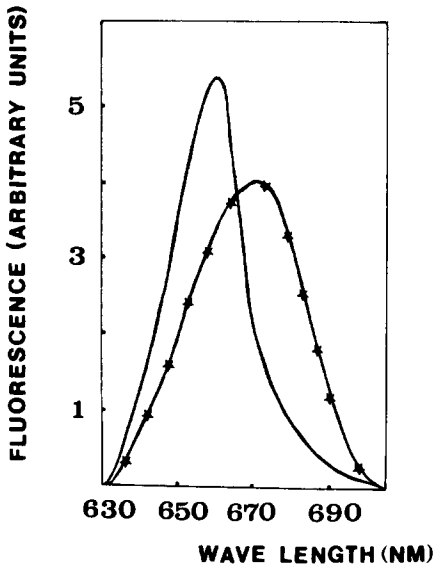

Fig.3. Emission spectra of pigmented liposomes before (–★–) and 5 min after (——) addition of α-latrotoxin (10^{-7} M) with Ca^{2+} (3 mM).

phosphatidylserine or PC/CL (1:1 molar ratio) (data not shown).

It has been clearly shown in Fig. 2 that Ca^{2+} enhanced the fusogenic effect of LTX. It should be stressed that the large unilamellar vesicles used do not fuse at this (0.12–3 mM) Ca^{2+} concentration. When LTX and Ca^{2+} were simultaneously added to the suspension of vesicles, the extent of fusion was significantly increased. The rate of fusion is dependent on the concentration of Ca^{2+}. It should be kept in mind that all the buffers

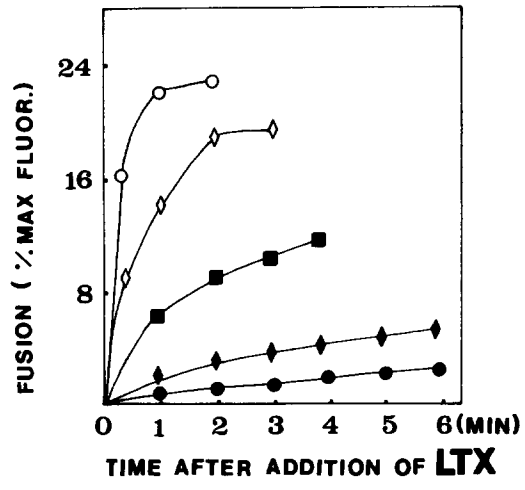

Fig.2. Enhancement of α-latrotoxin-induced fusion of liposomes as a function of Ca^{2+} concentrations. (●) LTX, without Ca^{2+}; (♦) LTX + 0.1 mM Ca^{2+}; (■) LTX + 1 mM Ca^{2+}; (◇) LTX + 2 mM Ca^{2+}; (○) LTX + 3 mM Ca^{2+}. Concentration of added LTX was 10^{-7} M. The maximum fluorescence (at 100% fusion) was set by lysing of Tb-containing vesicles with 0.5% sodium cholate in the presence of dipicolinic acid without EDTA.

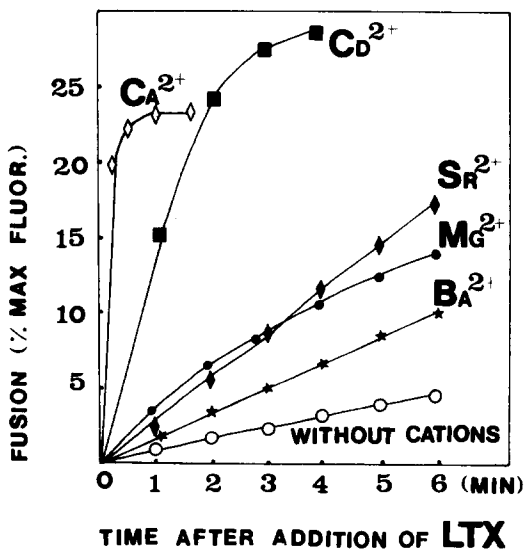

Fig.4. Effect of divalent cations (3 mM) of the time course of α-latrotoxin-induced fusion of liposomes. Concentration of added LTX was 10^{-7} M. The maximum fluorescence (at 100% fusion) was set by lysing of Tb-containing vesicles with 0.5% sodium cholate in the presence of dipicolinic acid without EDTA.

used in these experiments contained 0.1 mM EDTA, therefore the actual free Ca^{2+} concentration was respectively lower. It will be reasonable to suppose that LTX as a synexin [11] lowers the threshold of Ca^{2+} concentration required for the fusion of vesicles.

Fusion of phospholipid vesicles has been also studied by monitoring of liposomic membrane intermixing. The data obtained by this method supported the results of Tb-dipicolinic acid assay, but the sensitivity of this method did not allow one to make the kinetic fusion measurements. LTX in the presence of Ca^{2+} (3 mM) induces effectively the fusion of pigmented liposomes with non-pigmented ones and the reduction in energy transfer from Chl *b* to Chl *a* was observed (Fig. 3).

The synergism of the fusion effect of LTX and Ca^{2+} was also demonstrated by the method of laser correlation spectroscopy. According to these data the average diameter of the vesicles after incubation with LTX and Ca^{2+} (3 mM) increased from 170 nm to 260 nm.

The efficiency of other divalent cations in the enhancement of LTX-induced fusion of acidic liposomes has been shown in Fig. 4. The extent of fusion in the presence of Ca^{2+} was higher than that with other divalent cations. It appears that the sequence of cation efficiency for LTX-induced fusion was $Ca^{2+} > Cd^{2+} > Sr^{2+} > Mg^{2+} > Ba^{2+}$.

The participation of proteins in membrane fusion in various systems has been reported [12]. A number of proteins and peptides such as synexin [11], clathrin [13], diphtheria toxin [14], tetanus toxin [15] have been found to induce fusion of liposomes and their fusion mechanism has been studied. The results have proved that protonation, change in conformation, exposure of hydrophobic segments of proteins, and their insertion into the membrane definitely contribute to the fusion of liposomes.

The key finding reported in this paper is that LTX promotes the fusion of negatively charged vesicles and this effect is enhanced by Ca^{2+} and other divalent cations. It would be of particular interest to see the physiological relevance of the phenomenon.

The investigation on the mechanism of LTX fusogenic effect is now in progress.

REFERENCES

[1] Tzeng, M.C. and Siekevitz, P. (1979) in: Advances in Cytopharmacology, vol. 3, pp. 117–127, Raven Press, New York.
[2] Baba, A. and Cooper, J.R. (1980) J. Neurochem. 34, 1369–1379.
[3] Sokolov, Y.V., Chanturia, A.N. and Lishko, V.K. (1987) Biochim. Biophys. Acta 900, 295–299.
[4] Lishko, V.K., Terletskaya, Ya.T. and Gegelashvili, G.K. (1986) Doklady Academy of Science USSR 288, 740–743 (in Russian).
[5] Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468.
[6] Grebinozhko, E.I. and Nikolaenko, A.N. (1987) Ukr. Biochim. J. 59, 93–97 (in Russian).
[7] Szoka, E. and Papahadjopoulos, D. (1978) Proc. Natl. Acad. Sci. USA 75, 4194–4198.
[8] Lebedev, A.D., Levchuk, Y.N., Lomakin, A.V. and Noskin, V.A. (1987) Laser Correlation Spectroscopy in Biology (in Russian), Kiev.
[9] Wilschut, J., Düzgünes, N., Fraley, R. and Papahadjopoulos, D. (1980) Biochemistry 19, 6011–6021.
[10] Gad, A.E. and Eytan, G.D. (1983) Biochim. Biophys. Acta 727, 170–176.
[11] Hong, K., Düzgünes, N. and Papahajopoulos, D. (1981) J. Biol. Chem. 256, 3641–3644.
[12] Strittmatter, W.J., Couch, Ch.B. and Mundy, D.I. (1985) in: Membrane Fluidity in Biology, Cellular Aspects, vol. 4 (Aloia R.C., Boggs, J.M. eds) pp. 259–291, Academic Press, New York.
[13] Maezawa, Sh., Yoshimura, T., Hong, K., Düzgünes, N. and Papahajopoulos, D. (1989) Biochemistry 28, 1422–1428.
[14] Cabiaux, V., Vandenbranden, M., Falmagne, P. and Ruysschaert, J.-M. (1984) Biochim. Biophys. Acta 775, 31–36.
[15] Cabiaux, V., Lorge, Ph., Vandenbranden, M., Falmagne, P. and Ruysschaert, J.-M. (1985) Biochem. Biophys. Res. Commun. 128, 840–849.