Blocking of the β-Latrotoxin Channels in Bimolecular Lipid Membranes by Antibodies

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Abstract. Asymmetric ion channels are formed in a bimolecular lipid membrane by β-latrotoxin (LT) introduced to one (cis) side of the membrane. LT-specific antibodies added to the opposite (trans) side of the membrane block the current through the LT channels when a negative potential is applied to the cis side, no blockade is observed at positive potentials. LT-specific antibodies do not block the channel current when added to the cis compartment after removal of LT. LT-unspecific immunoglobulins have no influence on LT channel conductance.

Key words: β-latrotoxin — Ion channel — Antibody — Blocking — Lipid membrane

Introduction

Antibodies (Ig) are known to modify in some cases the properties of biological membranes (Turner 1981). Essential functions of biomembranes include passive ion transport through channels. However, almost no quantitative data are available on direct effects of immunoglobulins on currents through ionic channels, although this question is of great interest for the elucidation of the mechanisms by which antibodies influence biomembranes. In the present work the action of immunoglobulins on ionic channels in bimolecular lipid membranes were studied. β-latrotoxin (LT), a constituent of the venom of the spider Latrodectus tredecimguttatus, was used as the channel-forming agent.

Abbreviations: I—V, current-voltage; Ig, antibodies; LT, β-latrotoxin; Tris, 2-amino-2-hydroxy-methyl-propane-1, 3-diol.
Materials and Methods

LT was isolated from the venom of the spider *Latrodectus tredecimguttatus* by a procedure described earlier (Salikhov et al. 1982). The homogeneity of the neurotoxin was checked by disc-electrophoresis in polyacrylamide gel as well as by the analysis of both amino acid composition and terminal amino acids.

The immune serum containing antibodies against the given neurotoxin was obtained by immunization of experimental animals (rabbits) into lymph nodes at a dose of 25 μg/kg. The serum obtained had an antibody titre of 1:4000. Ig were isolated by the immunosorbent technique (porous cellulose beads immobilized with LT served as the carrier) (Gurvich and Lekhtchind 1981).

In control experiments, the immunoglobulin fraction of the blood serum of non-immunized rabbits was used; it contained no specific antibodies against LT.

Bimolecular lipid membranes were formed of 95% azolectin and 5% cholesterol by the method according to Montal and Mueller (1972) on an 0.1 mm aperture in the Lavsan partition dividing a teflon cell into two compartments. The cell was filled with aqueous solution, of 0.1 mol.l⁻¹ KCl/5 mmol.l⁻¹ Tris-HCl (pH 7.4), at 22 ± 2°C. The specific BLM conductance and capacity were 0.1 ± 0.05 mS/m² and 9 ± 1 mF/m², respectively.

The transmembrane current was measured by means of a Keithley 301 amplifier. For the determination of current-voltage (I—V) characteristics, membrane voltage was decreased at a constant rate (1.5 mV/s). The voltage sign is given for the compartment in which LT was present. The I—V characteristics did not depend on the direction of membrane voltage scanning.

After BLM formation, 4 ± 1 mg/l LT and 0.5 — 1.5 g/l immunoglobulins were added to the cell. A peristaltic pump (LKB, Sweden) was used to exchange solutions in the cell; the membrane was not damaged by solution exchange.

The number of LT channels incorporated in different experiments slightly varied. The differences being not significant. The experimental data presented were obtained from 10 different membranes.

Results

**Effect of β-latrotoxin on BLM.** Fig. 1 shows I—V characteristic of BLM in the presence of LT at one side of the membrane. The current jumps seen in the Figure were due to random closing and opening of the LT channel during membrane voltage scanning. For the lipid composition used, membrane I—V characteristics averaged over a great number of channels differed little from the linear one for the voltage interval studied (−70 mV to +70 mV).

The LT channels in BLM kept functioning throughout the observation period (over 1 h) after the LT containing solution in the cell was replaced by a LT-free one. This suggests a firm binding of the channels to the membrane (Finkelstein et al. 1976; Krasilnikov et al. 1982; Robello at al. 1984).

**Effect of immunoglobulins on BLM.** Control experiments showed that LT-specific antibodies and nonspecific immunoglobulins, when added to one or both compartments at concentrations between 0.5 and 1.5 g/l, did not increase the conductance of nonmodified BLM.
Effect of LT-nonspecific immunoglobulins on LT channels. LT was added to the cell at one side of the membrane. After the ionic channels had incorporated into BLM, 0.1 — 1.5 g/l of nonspecific immunoglobulins were added to the opposite side of the membrane. After thorough mixing, I—V characteristics were determined. The addition of nonspecific immunoglobulins had no effect on the I—V characteristics of the membrane: The characteristics were linear over a voltage range between −70 and +70 mV throughout the observation (30 min.). Immunoglobulins added to the compartment confining LT had no effect on the I—V characteristics either. Hence, nonspecific immunoglobulins have no effect on LT channel conductance.

Fig. 1. The current-voltage characteristic of BLM in the presence of $4 \pm 1$ mg/l LT at one side of the membrane. The membrane voltage was lowered at a rate of 1.5 mV/s.

Effect of LT-specific antibodies on LT channels. Fig. 2 shows I—V characteristic of BLM in the presence of both LT at one (cis) side and LT-specific Ig at the opposite (trans) side of the membrane.

The introduction of Ig isolated by the immunosorbent technique produced a decrease in the mean absolute value of transmembrane current at voltages below −20 mV. No marked effect of Ig on the ion current through LT channels was observed at positive voltages. Negative voltages resulted in characteristic current fluctuations at frequencies of about 3 Hz which were much higher than those in the absence of Ig (0.02 Hz). The block of ion current increased with the
Ig concentration at negative voltages. The plot in Fig. 3 shows the increase of the coefficient of transmembrane current blockade with the increasing antibody concentration (membrane potential $-50$ mV).

It is interesting to define at which side of the membrane the antibodies bind to LT channel to block the transmembrane current. To elucidate this question, an additional experiment was performed. First, LT was added at one (cis) side of BLM. The $I-V$ characteristics showed that LT had incorporated into the membrane and formed ion channels. Then, the LT solution was replaced by a solution containing 1.5 g/l Ig. LT was absent in the solution. The $I-V$ characteristics remained unchanged. No current blocking by Ig was observed. These observations permit the conclusion that specific antibodies block ion conductance of LT channels when in contact with the channel at the trans side of the membrane while having no effect on channel conductance when added at the cis side. Hence, LT incorporates into BLM asymmetrically.

Fig. 2. The current-voltage characteristic of BLM in the presence of both $4 \pm 1$ mg/l LT at the cis side of the membrane and LT-specific antibodies isolated by the immune sorbent technique at the trans side. The characteristic was obtained 10 minutes after the addition of immunoglobulins and solution stirring. The concentration of the antibody preparation was 1.5 g/l. The membrane voltage was lowered at a rate of 1.5 mV/s.

Fig. 3. Blocking of transmembrane current in the presence of $4 \pm 1$ mg/l LT at the cis side of BLM in dependence on the concentration of LT-specific antibody at the trans side. ($I_0$) is the transmembrane current in the presence of LT and in the absence of antibodies, ($I$) is membrane current following the addition of LT-specific antibodies to the cell. Four experimental values were obtained for four different membranes. Each point represents mean $\pm$ SEM. Membrane voltage was $-50$ mV.
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However in the latter experiment LT may not have been completely removed from perimembrane layers on solution replacement, and it may have prevented Ig from interacting with ion channels in the membrane. To rule out this possibility, a control experiment was carried out. Both LT and 1 g/1 Ig were added to both sides of the membrane. In this case, the current was blocked at both positive and negative membrane voltages greater than 20 mV. The Ig concentration used was thus excessive with respect to the LT concentration. No complete aggregation between Ig and LT did occur in the course of the experiment. A portion of Ig associated with ion channels in BLM and blocked them. Since excessive concentrations of Ig were used, LT may not have been removed completely. This is in support of the conclusion that LT-specific antibodies block ion channel conductance when added only from the trans side of the membrane.

The question arises whether the binding of specific antibodies to LT channel is tight enough. To answer this question, LT was added to the cis side of BLM, and 1.5 g/1 Ig was added to the trans side, and the I—V characteristics were determined (Fig. 2). Then, the solution was replaced by another without Ig present. The I—V characteristics remained unchanged for more than 20 min after the Ig removal. Thus, after the Ig removal, the current block persisted at negative voltages. This suggests that there is a tight binding of specific antibodies to LT channel.

It is interesting that LT formed no ion channels in BLM when the toxin had been preincubated with specific Ig for 5 hours.

Discussion

The results obtained suggest that LT-specific antibodies block the current through LT channels in a potential-dependent manner. Moreover, LT-nonspecific immunoglobulins have no influence on the conductance of LT channels.

Let us consider possible mechanisms for the blocking observed. It has been shown that blocking of the current through LT channels at negative potentials can be obtained by appropriately adjusting the lipid composition of BLM (Robello et al. 1984). It can be thus assumed that the blocking of transmembrane current observed in our experiments was due to the absorption of Ig on BLM. This interaction changes the phase state of lipids in the membrane. This would result in a potential-dependent blocking of the current through LT channels.

Such a blocking mechanism seems not to be the case since it would not explain the following facts: 1. No blocking of LT channel conductance is observed on adding Ig to the cis chamber from which LT had been removed. At
the same time, LT-specific Ig added to the trans side of the membrane block channel conductance. 2. LT-nonspecific immunoglobulins have no blocking effect on current through LT channel.

Another mechanism of transmembrane current blocking is more probable. LT ionic channels incorporate in BLM asymmetrically. At the trans-side the channel has an antigenic determinant of the same configuration as that in LT molecules during Ig synthesis. Moreover, this antigenic determinant is conformationally available for interaction with Ig which are present in the solution. LT-specific antibodies bind specifically to the antigenic determinant, and block the transmembrane current.

Some mechanisms for current blocking upon Ig binding to the LT channel may be suggested.

1. Possibly, the antigenic determinant is near the channel gate. An antibody molecule bound to this determinant may have a rotational or vibrational degree of freedom. The antibody closes the channel gate for some time while rotating or vibrating due to heat motion. This results in ionic current inhibition.

2. While binding to the LT channel the antibody changes the dipole or another electric moment of the channel gate. This can result in a deviation of the I—V characteristic of the membrane from linearity.

3. An antibody bound to the LT channel changes the phase state of phospholipids occurring near the channel. This, in turn, can result in current blocking at negative voltages.

The above suggestions can be useful in further studying the mechanisms of antibody-induced blocking of the current through ion channels.

It is quite possible that the ability of LT-specific antibodies to bind to ion channels at the trans side of the membrane only, and therewith to change ion conductance, reflects a general property of channel-specific antibodies.

The presence of the antigenic determinant at the LT channel makes us suggest that antibodies are synthesised by B Lymphocytes directly on ion channels which LT can from in the lymphocyte membrane.

The evidence obtained has shown that the study of ion channels using antibodies is a promising method in protein membrane structure investigation. Our findings can be also useful in developing new drugs against the venom of the spider *Latrodectus tredecimguttatus*.

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References


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