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Dependence of the conductance of the α-latrotoxin channel on applied potential and potassium concentration

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α-Latrotoxin, with a molecular weight 130 000, is the main component of black widow spider venom, and acts at the presynaptical level, inducing a notable release of neurotransmitters in the synapses of all vertebrates. In artificial lipid membranes, this neurotoxin induces the formation of cation-selective ionic channels, whose conductance depends on the intensity and direction of the applied potential. In fact, also in the presence of symmetrical solutions of potassium chloride, the voltage–current characteristics of the single channel strongly rectify. Such rectification, which depends on the concentration of the ions in solution, can be described by a one-site, one-ion model for a channel. The data fit provides the values of the three parameters describing the model. Moreover, a statistical analysis of the amplitude of the single channel, as a function of the concentration of potassium chloride, has made it possible to verify the consistency of the model used.

Introduction

Understanding the molecular mechanisms that regulate the flow of information through a cellular membrane is one of the most complex problems faced by molecular biology.

This involves, among other things, the study of the ionic transport mediated by proteins acting as channels. An important class of these proteins is that of presynaptic neurotoxins, which heavily interact with neurochemical transmission. This class includes α-LaTx purified by the venom of the Italian black widow spider (*Latrodectus mactans tredecimguttatus*). The toxic effect of this neurotoxin has been described for neuromuscular junctions in the frog, where α-LaTx triggers an increase in the frequency of miniature end-plate potentials [1], for synaptosomes, where it produces a notable release of neurotransmitters [2], and for a cloned neurosecretory cell line (PC12), where α-LaTx activates exocytosis and increases the concentration of cytoplasmic calcium [3]. By using the patch-clamp technique in PC12 cells, it has been shown that α-LaTx induces the formation of channels which are permeable to various cations [4]. The in vivo action of this neurotoxin is still unknown, even though various hypotheses have been considered, and might involve other mechanisms not related to the ion transport. Fusogenic properties have recently been verified for the α-LaTx part that protrudes from an artificial lipid membrane (BLM) [5]. This result suggests that α-Latrotoxin might favour the fusion of the vesicles containing neurotransmitters when it protends from the cytoplasmic part of the presynaptic membrane. Moreover α-LaTx could trigger the phosphatidylinositol breakdown observed in PC12 cells after treatment with this toxin [6]. In model systems and in PC12 cells, the fact that α-LaTx induces the formation of cation-selective and voltage-dependent channels [7,8] leads one to assume that this is a possible way in which the toxin, after binding to a cellular membrane, triggers its neurotoxic mechanism. In fact, by using specific antibodies, it has been possible to show that α-LaTx loses both its secretagogue property in PC12 cells and its capability of inducing channels in artificial lipid membranes [9].

In this paper, I have studied the electrical properties of the channels produced by α-LaTx in BLMs at different potassium chloride concentrations. The data, characterized by the rectification of the conductance dependent on ion concentration, are interpreted according to Eyring's rate theory. The experimental data are fitted by using a simple model based on three parameters, and the results are compared with those obtained for divalent ions [10].

Abbreviations: α-LaTx, α-latrotoxin; BLM, black lipid membrane.

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Materials and Methods

The membranes were obtained with the Montal and Mueller technique [11]. The double layers formed on a hole (about $3 \cdot 10^{-2}$ mm^2 in area) made in a Teflon partition 12.5 μm thick, which was placed between two compartments of the same material, each containing 2.2 ml of ionic solution. The membranes consisted of a mixture (1:1 molar ratio) of L-α-phosphatidylcholine (egg lecithin) and cholesterol (chol), dissolved in *n*-hexane (10 mg/ml). Both lipids were supplied by Calbiochem. The electrolytic solutions were prepared at different KCl concentrations, using RPE Carlo Erba salts, and were buffered to pH values of 7.5 and 5.5 with 10 mM Tris-HCl (Trizma; Sigma).

α-LaTx, purified by the venom of the Italian spider, *Latrodectus tredecimguttatus*, as previously described [12], was added to only one side of the membrane. This side has been defined as the *cis* side, while the other has been defined as the *trans* side.

The potential was applied with Ag|AgCl electrodes to the *trans* side, while the *cis* side was at virtual ground. Membrane formation was controlled by measuring its electric capacitance by means of a previously described circuit [10]. The voltage–current characteristics of the membranes in the presence of α-LaTx were obtained by stimulating the membranes with a triangular signal of peak-to-peak amplitude 200 mV and with a cycle of 100 s. This part of the circuit has also been detailed in a previous study [8]. All experiments were performed at room temperature (21–24°C).

The best fits of the experimental points were obtained by minimization programs running on a VAX 11/780 computer (Digital Equipment) [10].

Results

The effect of α-LaTx on artificial lipid membranes has been studied for various KCl concentrations at two different pH values. Addition of α-LaTx ($6 \cdot 10^{-10}$ M) to the *cis* side of a membrane of egg phosphatidylcholine and cholesterol (1:1 molar ratio) under voltage-clamp conditions causes an increase in the current that is typical of the formation of ionic channels. The formation kinetics of such channels, shown in Fig. 1, is similar to that already reported in previous papers [7,8,10]; it exhibits rare closing, and does not depend, in a significant way, on the concentration of the monovalent ions in solution.

The effect of KCl has been analyzed at various concentrations (10, 30, 100, 120, 200, 500 and 1000 mM) and at two different pH values (7.5 and 5.5). A statistical analysis of the amplitude of the unit current step as a function of KCl concentration has been made (Fig. 2).

Fig. 1. Current steps induced by α-LaTx in a lipid bilayer made of egg-phosphatidylcholine and cholesterol. Toxin was added to the *cis* side of the membrane (final concn. $6 \cdot 10^{-10}$ M) interposed between symmetrical solutions of 100 mM KCl buffered at pH 7.5 with 10 mM Tris-HCl. A 40 mV positive potential was applied to the *trans* side, while the *cis* side was at virtual ground.

A systematic study of the dependence of the conductance of the single channel on the applied potential has also been made. The I–V curves refer to membranes having only a few channels, and show the potential-dependence of the conductance of the single channel. In fact, the measurement sensitivity is such that the opening or closing of a channel can be directly observed.

The *I–V* characteristics rectify also when the membrane is placed between symmetrical solutions; such rectification depends on the ion concentration in the same way as in the presence of calcium and other divalent ions [10]. Table I gives the rectification ratio, *R*, defined as the ratio between the current values calculated at +90 and −90 mV, at different KCl concentrations at pH values of 7.5 and 5.5. *R* approaches 1 at high KCl concentrations, especially at a pH of 5.5. Such behaviour is shown respectively in Figs. 3 and 4, where the *I–V* curves are compared for different KCl concentrations (pH 7.5) and at two pH values

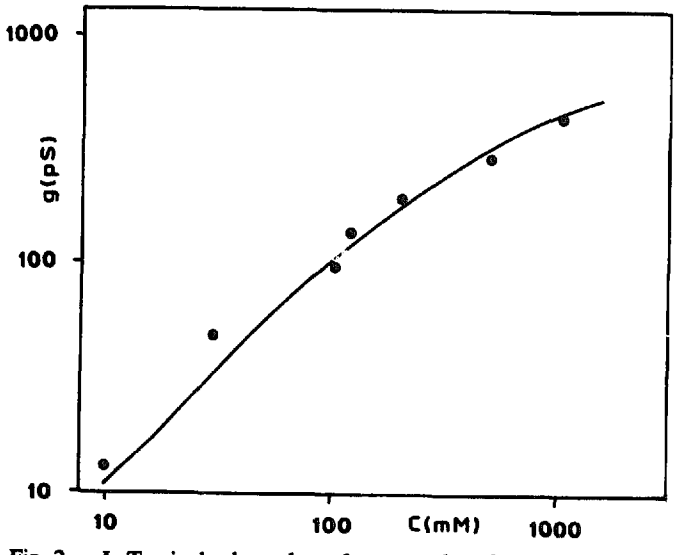

Fig. 2. α-LaTx single-channel conductance plotted vs. concentration of potassium chloride in a log-log scale. Each point is the average value of channel conductance found at +40 mV. The ionic solution was buffered at pH 7.5. The solid line was obtained from Eqn. 6, using the values of the parameters $g(0)_{max} = 830$ pS and $K = 1.3$ M^{-1}.

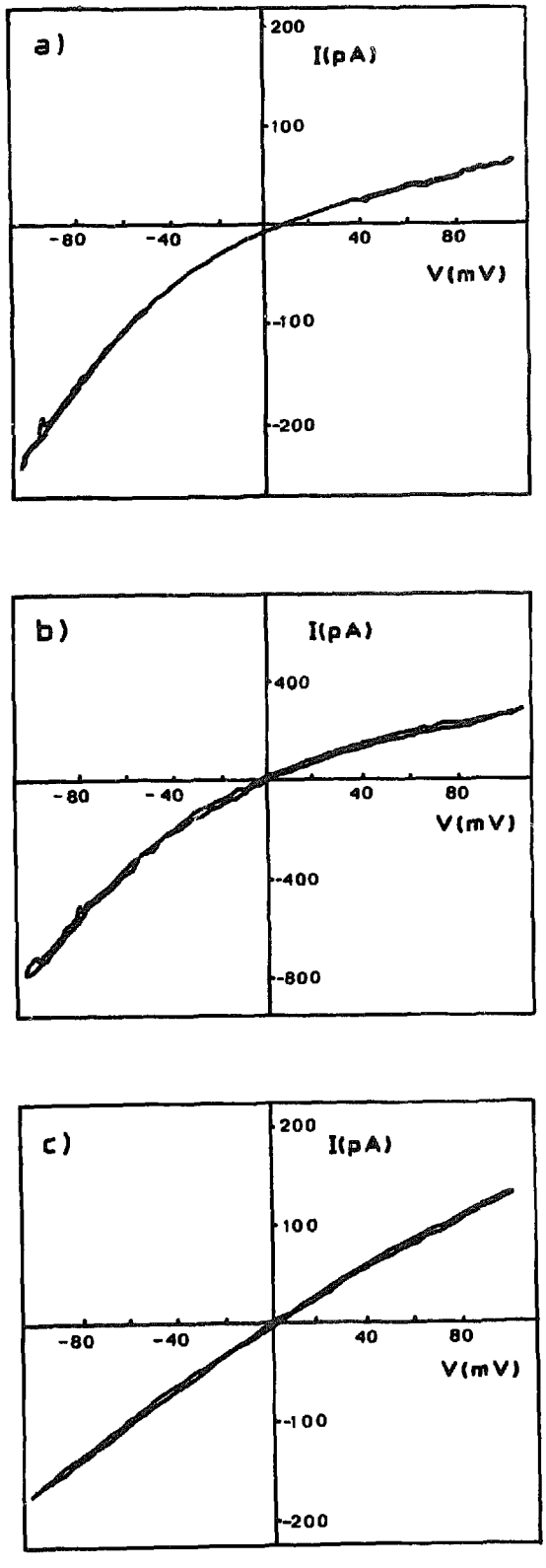

Fig. 3. Current–voltage characteristics after addition of the toxin to the *cis* side of a membrane interposed between symmetrical solutions of KCl buffered at pH 7.5: (a) 30 mM KCl; (b) 100 mM KCl and (c) 500 mM KCl.

(KCl = 200 mM). At high KCl concentrations, the curves are practically ohmic, whereas at low KCl concentrations, they are strongly asymmetric, since the current

TABLE I

Current rectification ratios as a function of potassium concentrations at pH 7.5 and 5.5

R is the ratio between channel current recorded at +90 and −90 mV of applied voltage. The current values were derived from the $I-V$ curves at different potassium concentrations at pH 7.5 and 5.5.

[K] (mM)	R (pH 7.5)	R (pH 5.5)
10	0.33	–
30	0.36	0.35
100	0.37	0.45
200	0.53	0.57
500	0.78	0.83
1000	0.76	–

flowing from the *trans* side to the *cis* is always much lower than the current flowing in the opposite direction. To explain this behaviour, a simple 'one-ion' model for a channel has been used; moreover, it has been possible to deduce the binding parameter, K, for the internal

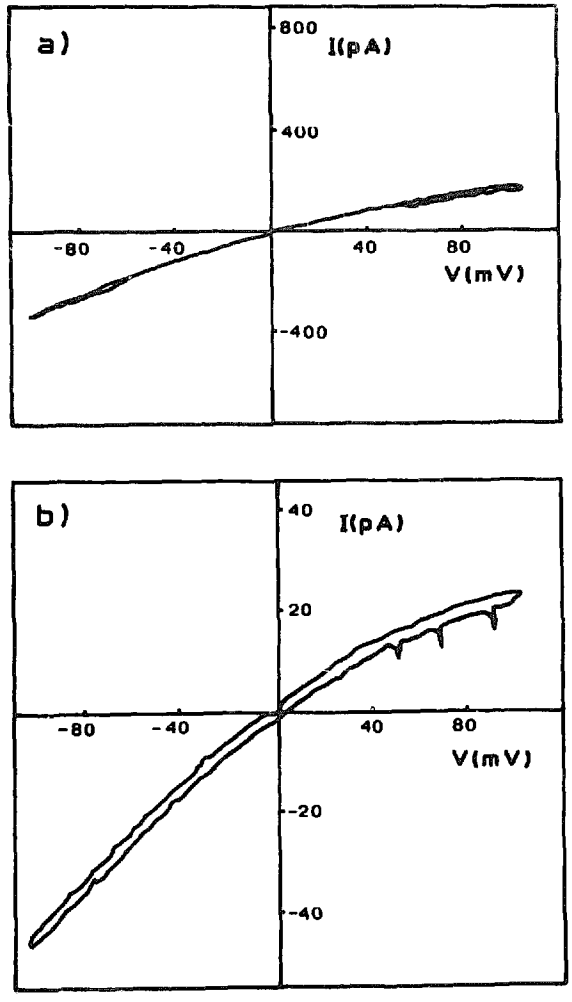

Fig. 4. Current–voltage characteristics for a membrane doped with α-LaTx (*cis* side) in the presence of 200 mM KCl buffered at pH 7.5 (a) and pH 5.5 (b).

site from the measurement of the conductance of the single channel at different KCl concentrations. In this way, the consistency of the model has been verified.

Theoretical model

All the experimental results can be explained by means of a one-ion model for a channel; such a model can be represented, in a schematic way, by two energy barriers separated by a binding site, with the barrier peaks lying midway between the internal site and the interfacial openings (Fig. 5). In accordance with Eyring's rate theory, considering monovalent cations in equal concentration, C, on the two channel sides, the pseudo rate constants for entering the channel are given by [13]:

$$\nu' = \bar{\nu}' C\, e^{\alpha u/2} \quad \nu'' = \bar{\nu}'' C\, e^{-(1-\alpha)u/2} \tag{1}$$

where u ($= u' = u''$) is the transmembrane potential in units of RT/F; F, R and T have the usual meaning; α is the electrical distance between the channel opening on the *cis* side and the internal site; $\bar{\nu}'$ and $\bar{\nu}''$ are parameters which are independent of the potential. The expressions for the rate constants for coming out of the channel can be written in an analogous way:

$$\mu' = \bar{\mu}'\, e^{(1-\alpha)u/2} \quad \mu'' = \bar{\mu}''\, e^{-\alpha u/2} \tag{2}$$

Introducing the following definitions: $\nu = \bar{\nu}''/\bar{\nu}'$ and $K = \bar{\nu}''/\bar{\mu}' = \bar{\nu}'/\bar{\mu}''$ (K = binding constant to the internal site), one can obtain the conductance of the single channel at a given potential, u [10,14]:

$$g(u) = \frac{F^2}{RT}\frac{\sinh(u/2)}{u/2} \times \frac{\bar{\nu}''C}{e^{-\alpha u/2} + e^{(1-\alpha)u/2} + KC(e^{\alpha u/2} + \nu\, e^{-(1-\alpha)u/2})} \tag{3}$$

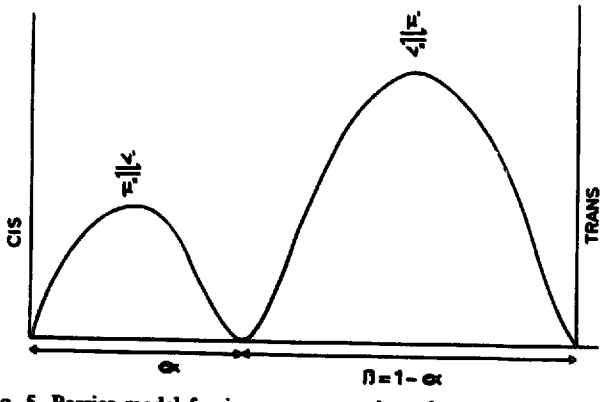

Fig. 5. Barrier model for ion movement through a one-site channel. The free-energy profile refers to the case of the zero applied field. The rate constants depend on the applied potential $u = u' - u''$ (where u' and u'' are, respectively, the potentials of the *cis* and *trans* sides) according to the Eyring rate-reaction theory.

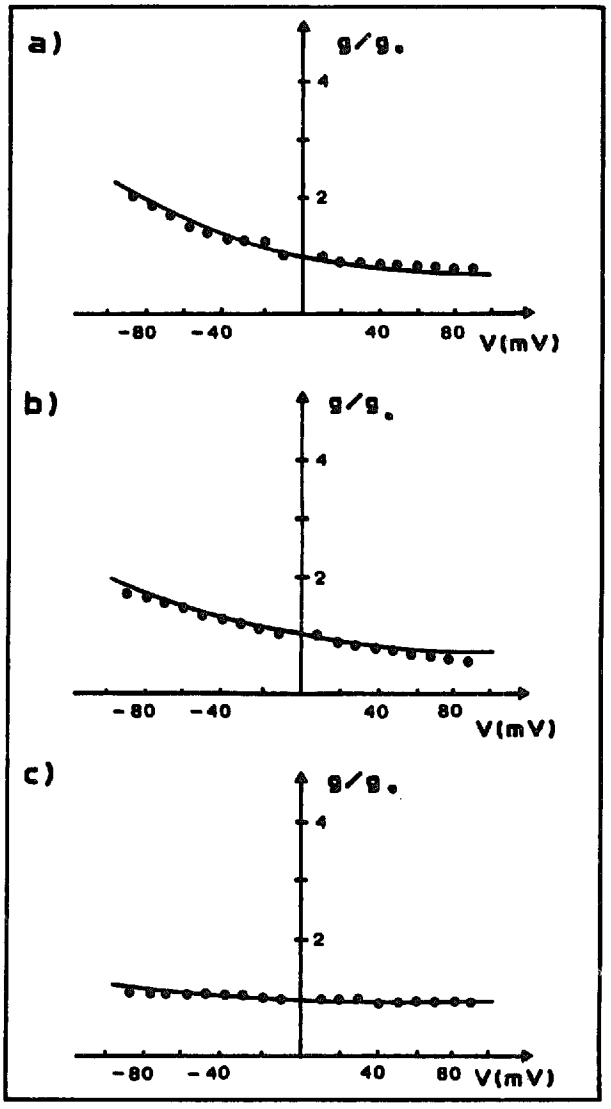

Fig. 6. Theoretical curves for the normalized conductance of the toxin channels in the presence of three solutions of KCl buffered at pH 7.5: (a) 10 mM KCl; (b) 100 mM KCl and (c) 500 mM KCl. The points were derived from the $I-V$ characteristics of Fig. 3. The solid lines were obtained from Eqn. 7 using the values of the parameters $\alpha = 0.3$, $\nu = 2.8$, $K = 1\ M^{-1}$.

The zero-potential conductance is given by:

$$g(0) = \frac{F^2}{RT}\frac{\bar{\nu}''C}{(1+\nu)(1+KC)} \tag{4}$$

At high ion concentrations, one can obtain the maximum conductance $g(0)_{max}$ of the channel:

$$g(0)_{max} = \frac{F^2}{RT}\frac{\bar{\nu}''}{K(1+\nu)} \tag{5}$$

Then the expression for $g(0)$ can be rewritten, and by simple calculations:

$$g(0) = g(0)_{max}\frac{KC}{1+KC} \tag{6}$$

This equation for the conductance of the single channel at zero potential contains the parameters $g(0)_{max}$, maximum conductance of the channel, and the binding constant K for the internal site.

By normalizing expression (3) to $g(0)$, one obtains the expression:

$$\frac{g(u)}{g(0)} = \frac{\sinh\left(\frac{u}{2}\right)}{u/2} \frac{(1+\nu)(1+KC)}{e^{-\alpha u/2} + e^{(1-\alpha)u/2} + (e^{\alpha u/2} + \nu e^{-(1-\alpha)u/2})KC} \quad (7)$$

which contains the parameters α, ν and K describing the model characteristics. It is worth noting that the value of K can also be obtained independently of Eqn. 6. Fig. 6 gives the experimental values of $g(u)/g(0)$ at different KCl concentrations at pH 7.5. The $g(u)$ values can be obtained directly from the experimental current vs. voltage curves, while $g(0)$ can be calculated by drawing the tangents to I–V curves at the origins and by estimating their slope. The continuous curves deduced from Eqn. 7 can be plotted by using the following three parameters:

$$\alpha = 0.3 \quad K = 1\ M^{-1} \quad \nu = 2.8$$

These values make it possible to fit all the experimental curves obtained at the different KCl concentrations. The continuous curve in Fig. 2 is the best fit of the experimental values of the single-channel conductance at different KCl concentrations. The curve deduced from Eqn. 6 can be plotted by using the following values for the parameters: $g(0)_{max} = 830$ pS and $K = 1.3$ M^{-1}. This value of the binding constant is in close agreement with the value obtained from the $g(u)/g(0)$ fit. The same procedure has yielded $g(0)_{max} = 520$ pS and $K = 1.7$ M^{-1} at pH 5.5.

Discussion

It is well known that α-LaTx, purified by black widow spider venom, acts on artificial lipid membranes by inducing the formation of ionic channels that are cation-selective and potential-sensitive. Insertion of this toxin occurs in a directed way [8] and seems to be facilitated by the presence of a receptor complex in the membrane [15].

The theoretical model used describes the electrical properties of the α-LaTx channel, in particular the current rectification depending on the ionic concentration. Although other mechanisms that would account for this phenomenon are possible [10], the model used seems to be simple enough and is able to fit several experimental data with a small number of parameters. Moreover, this model predicts that the conductance of the single channel (at a fixed applied potential) becomes saturated as the ion concentration increases. This has been experimentally verified, and the fit of the experimental values has provided the value $K = 1.3$ M^{-1}, which is close to the value obtained by fitting the data deduced, in an independent way, from the voltage–current characteristics. The amphibian Na^+ channel has been described by a similar theoretical model with four energy barriers [16]. Approaching the channel from the outside, there is a low barrier and then a well with an electrical distance of 0.27. This well is the binding site that produces saturation of the channel conductance with a binding constant of about 2.7 M^{-1}.

These values are comparable with the values obtained in our laboratory for the α-LaTx channel, confirming the pertinence of the model system used.

Furthermore, it is interesting to note that the values of the three parameters ($\alpha = 0.3$, $\nu = 2.8$, and $K = 1$ M^{-1}) are not very different from those obtained for divalent ions ($\alpha = 0.3$, $\nu = 1.3$, and $K = 1.5$ M^{-1}). The parameter, ν, is about twice as large, due to a difference in free energy (between the two peaks) of the order of 1 KT, which is about 4-times larger than the value obtained for divalent ions. Under similar experimental conditions, rectification is slightly less marked than for divalent ions. For example, at a 100 mM concentration of the ions in solution, the rectification ratio is equal to 0.37 as compared to 0.32 for calcium. This value seems to exclude the possibility for the rectification phenomenon to be due to a charge at the channel end. In this case, in fact, the ionic screening would be definitely larger in the presence of divalent ions. This mechanism is also ruled out by rectification measurements made as a function of pH. In fact, measurements made at pH 5.5 confirm that rectification is not very sensitive to the H^+ ion concentration; at this pH, α, ν and K are 0.3, 1 and 1.3 M^{-1}, respectively, and do not differ appreciably from those calculated at pH 7.5. Only the parameter ν seems somehow affected by a change in the H^+ ion concentration. Therefore, the effect of pH seems to be in the equalization of the barrier heights. However, if all the other parameters do not vary, the effect of pH on rectification is not very pronounced.

In contrast, the channel amplitude seems to depend significantly on pH (at least in the range considered). In fact, smaller conductance values have systematically been obtained, at all KCl concentrations, at pH 5.5 than at pH 7.5. The fit of the single-channel conductance as a function of the KCl concentration has provided a value of $g(0)_{max} = 830$ pS at pH 7.5. That is, about twice the value obtained at pH 5.5. This fact can explain the 50% reduction of the macroscopic conductance of a BLM doped with α-LaTx upon lowering the pH of the ionic solution from 7.1 to 5.2, as previously described [7]. The amplitude of the single channel seems, however, to depend on various parameters, including the microenvironment surrounding the toxin. Measure-

ments made on BLMs have yielded rather large values as compared with those obtained for PC12 cells [4]. On the other hand, measurements on BLMs containing acceptor molecules able to increase the affinity of α-LaTx for such membranes, have provided different values for the single channel [15]. It seems reasonable to postulate that, in all cases, differences in channel amplitude may be due to different lipid (or protein) compositions of the surrounding microenvironment. In fact, the effect of cholesterol contained in artificial lipid membranes on single-channel kinetics has already been described [8].

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