Electroporation and electropermeabilization of lipid bilayer membranes in the course of snakes’ venom intoxication

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ABSTRACT

The present study was undertaken to elucidate how the plastic properties of model membranes from native lipids of different tissues of rats change in the course of Macroviperia lebetina obtuse (MLO), Montivipera raddei (MR) and Naja kaouthia (NK) venoms processing. The presence of viper venom in organism lead to increasing of the electrical resistance of BLMs from liver and muscle lipids approximately on a sequence, while the BLMs from brain lipids has not shown a noticeable differences of plastic properties compare the control. The same concentration of cobra venom leads to decreasing of electrical resistance of BLMs from $10^{11}$ Ohm till $10^{8}$ Ohm. The low concentration of venom leads to appearance of channel activity. Especially it is noticeable in liver lipids in media of bivalent ions.

Keywords: Lipid Bilayer; Electroporation; Viper Venom; Breaking-Potential; Electropermeabilization

1. INTRODUCTION

As Vipers and pit vipers (snakes of the family Viperidae) produce venoms, which contain proteins that are normally part of the coagulation cascade, the normal haemostatic system and can cause tissue repair. Nevertheless human envenomations are very often accompanied by clotting disorders, hypofibrinogenenemia and local tissue necrosis. Although viperid venoms may contain well over 100 protein components, venom proteins belong to only a few major protein families, including enzymes (serine proteinases, Zn$^{2+}$-metalloproteinases, L-amino acid oxidase, group II PLA2) and proteins without enzymatic activity (disintegrins, C-type lectins, natriuretic peptides, myotoxins, CRISP toxins, nerve and vascular endothelium growth factors, cystatin and Kunitz-type proteinase inhibitors) [1,2].
venoms. The modeling of plastic properties of lipid bilayers in the interaction with venom could provide important information [17,18]. And as we thought, it was necessary to compare the results of these studies with analogous data from experiments with rats, processed by venom of Elapidae (Naja kaouthia, NK), containing the postsynaptic neurotoxin.

2. EXPERIMENTAL PROCEDURE

2.1. Tissue Processing

First, The venom of the Macrovi pare lebetina obtusa, Montivipera raddei and Naja kaouthia was tested for its ability to induce supramolecular changes in rats after short-term (10 min) intramuscular injection of the venom (0.35 mg/kg approx. 0.5 LD₅₀), by modeling of artificial membranes from native lipid content from some organs (liver, heart, brain and muscle). We tried to compare the data of in vitro and in vivo experiments. For in vitro experiments dried lyophilized toxin of MLO was dissolved in Tris-HCl buffer (pH 7.4) with a concentration of 3 × 10⁻⁵ M.

2.2. Phospholipid Processing

Lipids fractions were isolated from marked tissues of rats, according to the original Kates method [19]. Then a vacuum pump was used to remove the chloroform-methanol mixture. For in vitro experiments there were incubated with venom solution and held at a constant temperature of 37°C for 10 min. Then lipid sediments were dissolved in nonane (3% solution).

2.3. BLMs Formation and Measurements

The lipid bilayer membranes were formed from the lipid fractions of rat tissues on a teflon aperture by means of the Muller method [20]. A teflon cylindrical cup having a 0.8 mm hole is coupled to a glass chamber; so the cup separated two compartments filled with 5 ml electrolyte each. Electrical access to the baths was through a pair of Ag/AgCl electrodes.

Optical reflectance, electrical resistance and capacitance indicated the formation of planar lipid bilayers. The electrical parameters of the BLMs was determined on a device equipped with a Keithley 301 differential feedback amplifier (United States) in a voltage-fixation mode (current-clamp mode), which let to keep up a membrane potential on any level, independent from ionic streams. The potential setting on exit of generator completely falls on membrane, the resistance of which is much higher than that of electrodes, electrolyte and effective resistance of current’s gauge. Electrometric device can measure a current through membrane, under fixed value of transmembrane difference of potentials. 0.1 M KCl, NaCl, LiCl and KJ, KBr was served as ionic media.

The breaking-potential of membrane recorded in the experiments in shielded camera is taking as the threshold value of the voltage applied. Potential of membrane rapture is a criterion for valuation of natural defects of model membranes. Under electrical potential, radius of these holes is increasing. There is a critical value of radius (r₀), and when rₚ > r₀ membrane is destroying.

2.4. Statistical Analysis

For quantitative analysis of electrical parameters of BLMs, a Student’s test was used to compare differences at each time point, considering P < 0.05 as significant. All data were presented as mean ± SEM (n = number of experiments).

3. RESULTS

3.1. Electrical Current Measurements in Planar Bilayer Lipid Membranes (BLMs)

Electrical measurements of planar bilayers provide a means of measuring the changes in conductance, due to the formation of channels, pores or defects on the membranes, caused by the binding, insertion and translocation of peptides, at lowest concentrations, at which transient events can be detected. It also enables the determination of other features as: open probability, ionic selectivity of the pores and an estimative of pore’s size. Table 1 shows the results obtained with the in vitro action of three types of snake venom on the electrical conductance of BLMs from brain lipids of rats in media of K⁺ ions.

For the first series of experiment aliquots of 0.05 ml venom stock solution (concentration 10 mg/ml in Tris/HCl buffer, pH 7.4) was added to the both sides of BLMs formed from brain lipids in media with K⁺ ions.

<table>
<thead>
<tr>
<th>Table 1. Snake venoms in vitro action on the electrical properties of BLMs (resistance Rₘ, conductivity gₘ and breaking-potential Uᵢ) formed from brain lipids in media with K⁺.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td>Rₘ (Ohm)</td>
</tr>
<tr>
<td>gₘ (Ohm⁻¹)</td>
</tr>
<tr>
<td>Uᵢ (mV)</td>
</tr>
</tbody>
</table>

*Average value (±SEM) of minimum 7 different experiments.© 2012 SciRes. OPEN ACCESS
brane. After a few minutes of equilibration, –100 mV potential was applied in order to monitor changes in electrical properties of BLMs. Starting at 0.2 ml, venoms of MLO and NK showed dramatic changes of the membrane conductance (macroscopic integral conductance) and venom of MR showed significant activity (Table 1). An excess venom added in both sides of BLM raised sharply the membrane conductance and witnessed about cumulative mode of venom-membrane interaction. For the next series of experiment, the lipid fraction, after removing the chloroform-methanol mixture was incubated with venom stock solution and held at a constant temperature of 37°C for 10 min. Then venom solution was washed out and BLMs were formed from lipid mixture in nonane. This procedure didn’t lead to any significant changes in the means of conductance of bilayers induced by MLO and MR venoms, but BLMs from brain lipids of rats incubated with cobra venom showed a dependence of electrical resistance of bilayers from processing time (Figure 1) and venom concentration (Figure 2).

Collectively these results indicate that components of MLO and MR venom demonstrate rather surface activity during membrane-peptide interaction, while at least one or more components of cobra venom is definitely penetrate the bilayer. Indeed, single-channel current events induced by NK venom have been recorded in BLMs at lowest concentrations (<8 × 10⁻⁴ mg/ml).

### 3.2. Planar Lipid Bilayer Preparation for in Vivo Experiments and Ionic Permeability of BLMs

Planar lipid bilayers for these series of experiments were formed from solutions of native lipid mixtures from different tissues of rats (liver, heart, brain and muscle) after short-term (10 min) intramuscular injection of the venom (0.35 mg/kg approx. 0.5 LD 50).

The presence of viper venom in organism lead to the increase of the electrical resistance of BLMs from liver and muscle lipids approximately on a sequence, while the BLMs from brain lipids have not shown noticeable (Tables 2, 3). The same concentration of cobra venom leads to the decrease of electrical resistance of BLMs from 10¹¹ Ohm till 10⁸ Ohm. The low concentration of venom leads to the appearance of channel activity (Table 4). It is especially noticeable in liver lipids in media of bivalent ions.

### 4. CONCLUSIONS

As we can see from the experiments with planar lipid layers, that compare cobra venom with viper venom, peptides affinity is dominated by the electrostatic term. The hydrophobic effect is thus not sufficient to maintain a deep embedding of the peptides within the lipids. So the components of venom are very probably adsorbed at such a lipid interface with a few non-polar residues at the contact with lipid chain. This is in agreement with the data concerned the interaction of different peptides from snake venoms [21,22]. In such cases the binding to lipids

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**Table 2.** Snake venoms’ in vivo effect on the electrical properties of BLMs (resistance \(R_m\), conductivity \(g_m\) and breaking-potential \(U_i\)) formed from different tissue’s lipids in K⁺ medium.

<table>
<thead>
<tr>
<th></th>
<th>Brain</th>
<th>Heart</th>
<th>Liver</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(R_m) Ohm</td>
<td>(g_m) Ohm⁻¹</td>
<td>(U_i) mV</td>
<td>(R_m) Ohm</td>
</tr>
<tr>
<td>Control</td>
<td>1.3 × 10¹⁰</td>
<td>0.7 × 10⁻¹¹</td>
<td>355</td>
<td>1.3 × 10⁹</td>
</tr>
<tr>
<td><em>Vipera lebetina</em></td>
<td>5.6 × 10¹⁰</td>
<td>1.8 × 10⁻¹¹</td>
<td>309</td>
<td>6 × 10⁹</td>
</tr>
<tr>
<td><em>Vipera raddei</em></td>
<td>7.3 × 10¹⁰</td>
<td>1.3 × 10⁻¹¹</td>
<td>313</td>
<td>-</td>
</tr>
<tr>
<td><em>Naja kaouthia</em></td>
<td>7.4 × 10¹⁰</td>
<td>1.3 × 10⁻¹¹</td>
<td>407</td>
<td>2.3 × 10⁹</td>
</tr>
</tbody>
</table>

Each group contained 20 BLMs from four tissues. \(P > 0.01\) by Student’s t-test relative to the corresponding control.
Table 3. Montivipera raddei venom in vivo effect on the electrical properties of BLMs (resistance $R_m$, conductivity $g_m$, and breaking-potential $U_r$) formed from different tissue’s lipids in univalent ions media.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$K^+$</th>
<th>Na$^+$</th>
<th>Li$^+$</th>
<th>$J^-$</th>
<th>Cl$^-$</th>
<th>Br$^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>$7.25 \times 10^{10}$</td>
<td>$1.3 \times 10^{-11}$</td>
<td>313</td>
<td>$5.26 \times 10^{-10}$</td>
<td>$1.9 \times 10^{-11}$</td>
<td>239</td>
</tr>
<tr>
<td>Liver</td>
<td>$7.4 \times 10^{10}$</td>
<td>$1.4 \times 10^{-11}$</td>
<td>407</td>
<td>$4.2 \times 10^{-10}$</td>
<td>$2.4 \times 10^{-11}$</td>
<td>249</td>
</tr>
<tr>
<td>Muscle</td>
<td>$7.25 \times 10^{10}$</td>
<td>$1.3 \times 10^{-11}$</td>
<td>313</td>
<td>$5.26 \times 10^{-10}$</td>
<td>$1.9 \times 10^{-11}$</td>
<td>239</td>
</tr>
</tbody>
</table>

Each group contained 20 BLMs from three tissues. P > 0.01 by Student’s t-test relative to the corresponding control.

Table 4. Naja kaouthia venom in vivo effect on the electrical properties of BLMs (resistance $R_m$, conductivity $g_m$, and breaking-potential $U_r$) formed from different tissue’s lipids in univalent ions medium.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$R_m$ Ohm</th>
<th>$g_m$ Ohm$^{-1}$</th>
<th>$U_r$ mV</th>
<th>$R_m$ Ohm</th>
<th>$g_m$ Ohm$^{-1}$</th>
<th>$U_r$ mV</th>
<th>$R_m$ Ohm</th>
<th>$g_m$ Ohm$^{-1}$</th>
<th>$U_r$ mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>$7.4 \times 10^{10}$</td>
<td>$1.4 \times 10^{-11}$</td>
<td>407</td>
<td>$4.2 \times 10^{-10}$</td>
<td>$2.4 \times 10^{-11}$</td>
<td>249</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>$7.4 \times 10^{10}$</td>
<td>$1.4 \times 10^{-11}$</td>
<td>407</td>
<td>$4.2 \times 10^{-10}$</td>
<td>$2.4 \times 10^{-11}$</td>
<td>249</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

was also strictly curvature-dependent.

Studies following the changes in the plastic properties of membranes of different tissues during snake venom envenomation are scarce. The analysis of the results obtained in the experiments with whole venom is difficult because of the complexity of ongoing processes, each of which may induce a various cascades of tissue damages. At the same time an important task of such research is to provide not only opportunities to improve tissue recovery and clinical outcome of patients, but also to adopt novel therapeutic strategies to treat a number of diseases, such as different types of aggressive cancers [23] or neurological damages [24,25].

5. ACKNOWLEDGEMENTS

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REFERENCES


