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Effect of Triphenyllead Chloride on the Resting Potential and Electrical Conductance of *Nitellopsis obtusa* Membrane

Z. Trela, J. Radecki *, S. Przestalski

Agriculture University, Department of Physics and Biophysics, Norwida 25, 50-375 Wrocław, Poland.
* University of Agriculture and Technology, Chemistry Department, 10-957 Olsztyn-Kortowo, Poland.

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Abstract

The effect of triphenyllead chloride on membrane potential and electrical conductance of internodal cells of the alga *Nitellopsis obtusa* was studied. It was found that the compound studied, when present at 50 μM concentration, caused membrane depolarization and increased electrical conductance. The effect is, however, observed only after 0-30 min incubation, which may be due to the cumulation of triphenyllead in the cell wall before direct contact with the plasma membrane could occur. After 30 min incubation in triphenyllead solution the average relative changes in the membrane potential and electric conductance were -0.1 ± 0.04 and 0.33 ± 0.08 (n=12), respectively. These changes may be due to increased permeability of chloride ions brought about by the compound's interaction with channel proteins, or increased unspecific leakage of ions induced by a disturbance in the membrane structure.

Keywords: triphenyllead, membrane potential, membrane conductance, *Nitellopsis obtusa.*

Introduction

Organometallic derivatives of lead and tin are biologically active and constitute a toxic menace to the environment. From recent research it can be concluded that the compounds are membrane active, i.e. they exhibit hemolytic action [3, 4], modify the lipid phase of membranes [1], and have algae- and bactericidal effects [13, 2, 5].

Thus, the question arises whether or not the toxic effect of the organometallic compound's destructive effect on the channel mechanisms are responsible for electrophysiological phenomena; i.e. whether they affect channel proteins which control membrane potential. Oyama [9] found, for instance, that the neurotoxic effect of triphenyltin is connected with modulation of sodium channel function, which altered the current-voltage characteristics of the channel.

The objective of the present study was to determine the effect of a similar organolead compound - triphenyllead - on the electrophysiological parameters (membrane potential and electric conductance) of the plasma membrane of the internodes of the alga *Nitellopsis obtusa.*

Materials and Methods

Fresh internodal cells of *Nitellopsis obtusa* were used in the investigation. The alga was taken from the natural environment (Zagłębocze lake by Sosnowica, Chełm region) after being kept in a laboratory aquarium.

Plant isolated, single internodal cells (of mean dimensions: 0.43 mm diameter and 2.6 cm length) were incubated for 24 h in darkness in measurement solution (APW).

The control solution of pH 6.3 contained 1 mM NaCl, 0.1 KCl and 0.1 $CaCl_2$. The compound studied, triphenyllead, came from the ALFA Chemical Co. The incubation solution containing triphenyllead was prepared by dissolving the compound in a small amount of ethanol (100 μl) and then in APW. The same amount of ethanol was added to the control APW solution (100 μl ethanol to 200 ml APW). Triphenyllead concentration in the incubation solution was $5 \cdot 10^{-5}$ M in all experiments.

Membrane potential and electric resistance were measured with the electric system shown in Fig. 1.

Measurements were performed on a cell placed in a three-compartment container. The compartments (cells) were filled with liquid and partitioned by narrow

empty spaces that insured electric isolation between the compartments. The middle compartment was perfused with control solution or solution containing a modifier at a rate of 1 ml/min. In that part of the container current and membrane potential were measured. The middle compartment covered 3 mm of the alga cell.

Membrane potential was measured with a pair of glass microelectrodes (V_H and V_L) filled with 3 M KCl. In the current circuit Ag/AgCl electrodes were used.

The electric conductance of the modified membrane fragment was determined on the basis of the voltage response to a constant signal of ±(10-20) nA, 0.5 s duration and 15-20 s repetition time.

All measurements were conducted at room temperature (21±2)°C.

The analog measurement signals were digitally converted using an A/D conversion card, D/A (ADVANTECH Co., model PCL-812 PG, PC-LabDAS Data Acquisition Software PCLS-700) and saved on hard disk. Final elaboration of the measurement data was done in a spreadsheet.

Results and Discussion

The results of measurements of electric conductivity and membrane potential of *Nitellopsis obtusa* membrane modified with triphenyllead are presented in Figures 2 and 3.

The respective curves represent mean values from n=12 independent experiment of membrane potential (Fig. 2) and electric conductance (Fig. 3) as a function of time. In each experiments the cell was kept in the control solution (APW) for the first 60 min., and in APW + 50 μM Ph_3PbCl for the remainder (60-120 min).

The results presented in Figs. 2 and 3 mean that:

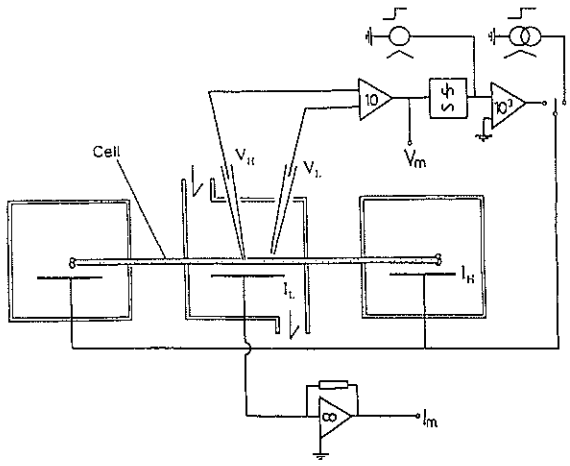


Fig. 1. Block diagram of measurement setup. V_H , V_L - voltage microelectrodes, I_H , I_L - current Ag/AgCl electrodes, V_M , I_M - analog signals of membrane voltage and current sent to data acquisition system.

1. The studied compound practically does not affect the membrane (or only slightly) during the initial period (0-20 min) of incubation in the trimethyllead solution.

2. The resting membrane potential (plasmalemma+tonoplast) undergoes depolarization in the presence of triphenyllead, as seen in Fig. 2. The mean change in the resting potential after 60 min of the compound's action (i.e. 60-120 min from the start of the experiment) was –0.1±0.04 (n=12), which corresponds to about 10 mV mean depolarization.

3. Electrical conductivity (plasmalemma+tonoplast), which tends to decrease in control solution (Fig. 3, 0-60 min), is increased by the modifier (Fig. 3, 60-120 min).

The increase in conductance, like depolarization, is especially visible after 30 min. Mean conductivity during 60 min incubation in APW decreased from G_0 = 0.49 Sm^{-2} to G_{60} = 0.43 Sm^{-2}, whereas after consecutive 60 min of incubation in triphenyllead solution it increased to G_{120} = 0.57 Sm^{-2}, the corresponding relative mean change being $(G_{120} - G_{60})/ G_{60}$ = 0.33±0.08 for n=12.

Although a majority of the cells examined reacted with depolarization and increased electric conductance of the membrane during 60 min incubation in 50 μM triphenyllead solution, some deviations from this rule were observed. For a small number of cells (2 from between 12) instead of increase in conductance a small decrease was observed, and slight (within statistical error limits) changes in the resting potential.

The effect of the studied organolead compound Ph_3PbCl on the internodal cells of *Nitellopsis obtusa* differs from the effect of the amphiphilic compounds studied earlier [10, 12] in the time courses of the quantities measured. Thus, while the addition of amphiphilic compounds to the incubation solution caused an immediate reaction of the cell in the form of resting potential and conductance changes, triphenyllead caused marked changes only after a certain incubation period.

This may be explained by the fact that the cell wall constitutes a barrier between the medium and plasma membrane. This wall may, in some cases, function as a kind of molecular filter, causing the lead alkyl derivative present in the medium to accumulate in the wall. The action on the plasma membrane occurs only after its concentration in the wall has risen to a certain level. Such circumstances could explain, to a certain degree, the lack of depolarization and of increased conductance for some cells. During 60 min incubation the triphenyllead present could accumulate in the wall only, without reaching the plasma itself.

Before such a hypothesis can be definitely stated, further investigation is needed, using techniques other than electrophysiological ones (e.g. quantitative analysis).

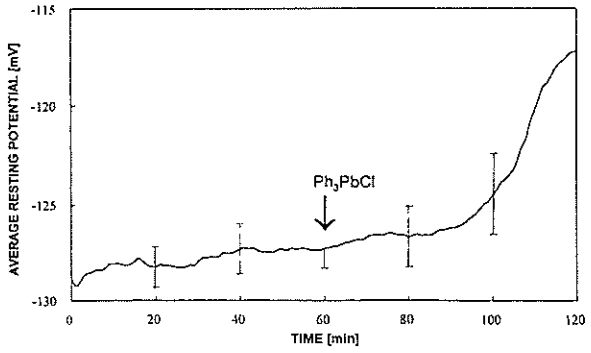


Fig. 2. Mean resting potential of 12 cells of *Nitellopsis obtusa* as a function of time. At time 0-60 min each of the cells was in control solution (APW), at time 60 min the incubation solution was repleaced with APW + 50 μM Ph3PbCl.

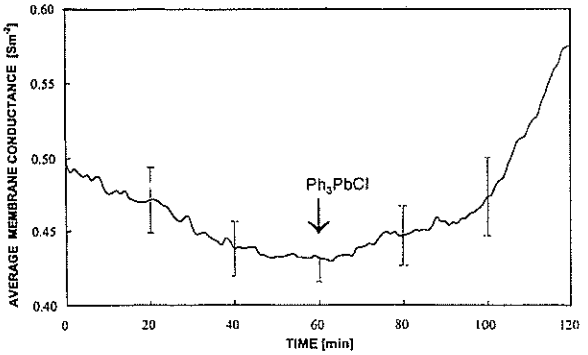


Fig. 3. Mean membrane conductance of 12 cells of *Nitellopsis obtusa* as a function of time. At time 0-60 min. each of the cells was in control solution (APW), at time 60 min the incubation solution was repleaced with APW + 50 μM Ph3 PbCl.

Changes in the resting potential and membrane conductivity induced by triphenyllead may suggest that the compound affects the transport processes responsible for the electrogenesis in cells of *Nitellopsis obtusa*. One may assume (with a measure of simplification) that:

1. The resting membrane potential E is mainly due to the electrodiffusive effect and can thus be expressed by the Goldman-Hodgkin-Katz equation:

$$E = \frac{RT}{F} \ln \frac{P_K K_o + P_{Na} Na_o + P_{Cl} Cl_i}{P_K K_i + P_{Na} Na_i + P_{Cl} Cl_o},$$

where R is the gas constant, T is the temperature, F the Faraday constant, and P_K, P_{Na} and P_{Cl} denote permeability coefficients of the ions K^+, Na^+, Cl^-, while K_o, and K_i etc. denote outer and inner concentration of the ions.

2. Membrane conductance is connected with diffusive fluxes of ions which depend on permeability coefficients P_K, P_{Na} and P_{Cl}. The observed, triphenyllead induced depolarization together with increased membrane conductance suggests that the studied compound causes an increase in the permeability of one or a number of the principal ions, the numerator of the Goldman-Hodgkin-Katz equation increasing more than the denominator (depolarization). Since in the case of the cells considered, $K_o < K_i$, $Na_o < Na_i$ and $Cl_o < Cl_i$, [6, 8], the results obtained may suggest the increased permeability of chloride ions. Thus, one can suppose that the positive triphenyllead ions, from dissociation of triphenyllead chloride, penetrate the plasma membrane and interact directly or indirectly with channel proteins responsible for the transport of chloride ions. Such interaction is possible by changing the transmembrane potential resulting from increased surface density of positive charges coming from the triphenyllead cations. This in turn may activate the voltage dependent chloride channels [7, 11]. The lowering of the absolute value of the transmembrane potential (depolarization) and increased membrane conductance may also result from the destructive effect of triphenyllead on the lipid phase of the membrane, which causes local loosening of its structure and consequently unspecific leakage of ions. In order to decide whether the effects observed result from the compound's direct interaction with the channel mechanisms or are accomplished via its effect on membrane lipids, further studies are needed, including measurements of current-voltage characteristics of modified membrane at various ionic compositions of the incubation medium.

Acknowledgments

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