Action of Lindane on the Current-Voltage Relationship in the Plasma Membrane of *Elodea* under Passive Conditions

Kurt Schefczik and Wilhelm Simonis

Universität Würzburg, Lehrstuhl Botanik I, Mittlerer Dallenbergweg 64, D-8700 Würzburg

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Side effects of the chlorinated hydrocarbon insecticide Lindane on the plasma membrane of the submerged macrophytic fresh-water plant *Elodea densa* were studied. Single glass microelectrodes were inserted into single cells and membrane potential, membrane resistance and electrode resistance were recorded using a new designed electrophysiological monitoring system ("ELM 2"). The current-voltage relationship in the plasma membrane of *Elodea* cells under passive conditions is nearly linear for relaxing current pulses up to 10^{-8} amperes. Lindane treatment, produced first (3.5 hours) a strong nonlinearity in current-voltage relationship with increased membrane resistance for inward current flow, and later (24.5 hours) an increased membrane resistance both for inward and outward current flow. It is discussed that the earlier reported lowering of the potassium selectivity in the plasma membrane of *Elodea* after Lindane treatment will be the reason for the alterations of current-voltage relationship.

The chlorinated hydrocarbon insecticide Lindane is still widely used for pest control. The toxicity of Lindane on insects is mainly based on alterations of nerve membrane properties [1]. Studies of Lindane action on synthetic phospholipid bilayer membranes support that chlorinated hydrocarbon insecticides may interact with membrane lipids [2]. Beyond that side effects of Lindane on cell membranes of water plants are possible. In a recent study we have described Lindane action on light-induced membrane potential changes and diffusion potentials in leaf cells of the water weed *Elodea densa* [3]. Now we wish to present data about the current-voltage relationship in the plasma membrane of Lindane treated Elodea leaf cells under passive conditions.

Experimental

Elodea densa was cultivated under laboratory conditions as described earlier [4]. *Elodea* shoots were equilibrated for 24 to 36 hours (25 °C, 10 W × m^{-2} white light) in a solution called APW 6.5 (0.1 mM KCl, 1 mM NaCl, 0.05 mM $CaSO_4$, 2 mM NaH_2PO_4, adjusted to pH 6.5 with Na_2HPO_4) and then incubated in APW 6.5 adding 20 ppm Lindane (gamma-1,2,3,4,5,6-hexachlorocyclohexane, 6.9×10^{-5} M) dissolved in methanol (final concentration 0.1%) for Lindane treatment under the conditions of equilibration or adding methanol only for control experiments, respectively. After pretreatment, single leaves were transferred into a perfusion chamber for electrophysiological experiments [5], being continuously washed with APW 6.5 plus 10^{-3} M NaCN and ± Lindane, respectively.

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Electrophysiology

Single electrodes, inserted into single cells, served both for current injection and potential recording. A new designed monitoring system, described as "Electrophysiological Monitor" (ELM 2) by Schiebe, Jäger, and Pauschinger [6, 7], was used to record the membrane potential, the membrane resistance and the electrode resistance simultaneously and continuously. The monitor makes use of the frequency dependence of the voltage devider network which is constituted when the electrode is inserted into the cell [6–9]. The equivalent electrical circuit consists at least in the electrode resistance R_e in parallel with the small stray capacitance C_e of the electrode, connected in series with the membrane resistance R_m paralled by the large membrane capacitance C_m. The different time constants $\tau_m = R_m \times C_m$ and $\tau_e = R_e \times C_e$ of the network could be separated sufficiently for τ_m/τ_e was greater than about 50 [7]. τ_e was minimized by carefully capacitance compensation in the feedback loop of the preampli-

fier. The current stimulus consists in a combination of short and long relaxing rectangular pulses, which are adjusted in their width to the small time constant of the electrode (short pulse) and the great time constant of the membrane (long pulse). The voltage response of the circuitry was analysed by the "Electrophysiological Monitor" by sampling the signal amplitudes and computing the membrane potential and membrane resistance together with the electrode resistance.

In the presented experiments the system was applicated successfully for measuring electric parameters in plasma membranes of plant cells for the first time. The membrane resistance data measured with this system are in good agreement with measurements using separate electrodes for current injection and potential recording [10].

Results and Discussion

Lindane action on plant metabolism and growth are described repeatedly [11 – 13]. At present there is some evidence for distinct targets of Lindane action in living plant cells. Simonis and Lee [14] have shown selective actions of Lindane on the blue-green alga *Anacystis nidulans*. In an earlier study we have described the plasma membrane as a target for Lindane action in living plant cells [3]. This finding can be supported now by presenting the current-voltage characteristic in plasma membranes of Lindane treated *Elodea* leaf cells under passive conditions. In an inexcitable membrane in the passive state (without electrogenesis) the slope in the current-voltage (I-E) characteristic indicates the resistivity of the membrane [15]. The membrane resistance is limited by the ionic gate with the highest conductivity. Elimination of electrogenesis in *Elodea* cells was achieved by darkening (blocking of photophosphorylation) and adding of cyanide (blocking of oxidative phosphorylation). Voltage responses on depolarising and hyperpolarising relaxing current pulses were analysed in current steps of 2×10^{-9} amperes up to 10^{-8} amperes (Fig. 1). Using small currents, the I-E relationship of the intact membrane is nearly ohmic as it is expected under passive conditions ([15], Fig. 1, graph C). Contrary to graph C the I-E characteristic of the membrane shows a clear nonlinearity after 3.5 hours Lindane treatment (Fig. 1, graph L_1). The diode characteristic of graph L_1 indicates a lowering in the ionic conductivity in particular for the inward current flow. Together with this the diffusion potential is decreased similarly as described earlier [3]. Finally, after 24.5 hours of Lindane treatment (Fig. 1, graph L_2), the membrane rectification characteristic is nearly disappeared and the membrane resistance is high both for inward and for outward current flow. Under passive conditions the membrane potential is mainly a potassium diffusion potential and therefore the potassium pore has the highest conductivity [5]. Lindane decreases the potassium conductivity in the plasma membrane of *Elodea* under passive conditions [3]. This can now be seen directly in the I-E characteristic. The different slopes for outward current flow in the I-E

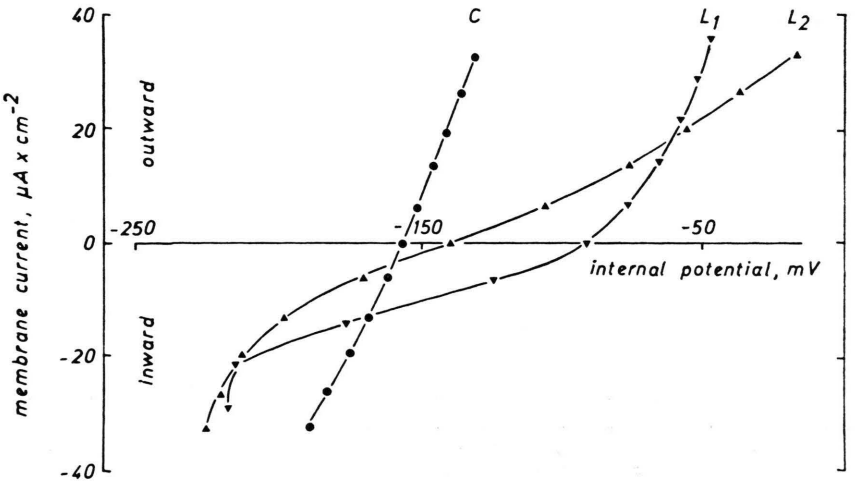

Fig. 1. Current-voltage relationship in the plasma membrane of *Elodea* leaf cells in the darkness in APW 6.5 plus 10^{-3} M NaCN (passive conditions) after different pretreatment periods with 20 ppm (6.9×10^{-5} M) Lindane. The membrane currents were calculated without respect of cell coupling. C: Control (19.5 hours), L_1: 3.5 hours Lindane treatment, L_2: 24.5 hours Lindane treatment. See text for further explanation.

relationship after 3.5 hours and 24.5 hours of Lindane treatment, respectively (see Fig. 1, graph L_1 and L_2) indicate that the outward current flow is slower affected than the inward current flow by Lindane treatment. We assume therefore that the external applied insecticide first may have acted on the outside of the plasma membrane and so first decrease inward current flow. The *Elodea* leaf is a tissue built up by electrically coupled cells [16]. Therefore the presented experiments cannot clarify if the measured enlargement in the membrane resistance caused by Lindane treatment is only the result of increased membrane resistance or additionally the result of lowering in the cell coupling. Further experiments are in progress.

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