Organolead Toxicity in Plants: Triethyl Lead (Et_3Pb^+) Acts as a Powerful Transmembrane Cl^-/OH^- Exchanger Dissipating H^+-Gradients at Nano-Molar Levels

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Triethyl lead (Et_3Pb^+), a highly toxic oxidation product of the anti-knock agent tetraethyl lead (Et_4Pb) was shown to act as anion (Cl^-/OH^-) antiporter in plant membranes, dissipating energy-dependent ion gradients, membrane potentials, and consequently turgor. This mechanism was demonstrated with tonoplast-type vesicles isolated from coleoptiles of *Zea mays L.* The ATP-driven H^+ accumulation within those vesicles was abolished already at nano-molar levels of Et_3Pb^+, but only in the presence of Cl^-.

In intact cells the turgor dependent indole-3-acetic acid induced elongation growth of coleoptile segments of *Avena sativa L.* was inhibited by Et_3Pb^+ at micro-molar levels and after a lag of 15–20 min. This lag might be due to a slow penetration of the agent through the waxy cuticle and the cell wall.

Introduction

Tetraethyl lead (Et_4Pb) is used as anti-knock agent in motor fuel. Its degradation product triethyl lead (Et_3Pb^+) is toxic to cells of mammalian origin [1–5] as well as of algae and higher plants [3, 6–9]. Recently, triethyl lead was suggested to be one of the factors causing progressive damage of European forests [10–12] (but see [13]). The toxic effect of Et_3Pb^+ to cells was attributed to an inhibition of microtubule assembly [2, 3, 5]. In *in vitro* experiments it has been found that Et_3Pb^+ (>1 μM) interacts with thiol groups present in tubulin dimers. As a result tubulin looses its capability for microtubule assembly [4]. In the present study, evidence will be given that in plant cells, demonstrated with isolated vacuolar vesicles from *Zea mays L.*, Et_3Pb^+ also acts as a potent trans-membrane Cl^-/OH^- exchanger. Thereby it dissipates ion gradients at nano-molar concentrations, *i.e.*, a range which is 1000-fold lower than that affecting microtubules [4].

Materials and Methods

The preparation of microsomal and tonoplast vesicles from coleoptiles of *Zea mays L.*, and the separation of membrane fractions by density gradient centrifugation was performed according to [18, 21]. The ATP-dependent intravesicular acidification of tonoplast-type vesicles was demonstrated with a dual wavelength method and neutral red (40 μM) as pH indicator [15, 19, 21]. Et_3Pb^+ and Et_4Pb were purchased from Ventron, Karlsruhe, FRG.

Results and Discussion

The energy-dependent transport of ions and solutes into the vacuole of a plant cell (necessary for the formation of turgor) can be studied by using isolated vacuoles or membrane vesicles derived from the tonoplast (reviewed in [14]). The primary driving force for the accumulation of osmotic compounds within the vacuole or vacuolar vesicles was shown to be an ATP-dependent H^+-pump [15]. A second, pyrophosphate-driven H^+-pump localized at the tonoplast was demonstrated only recently [16–18]. The transport of H^+ strictly depends on a cotransport with Cl^- [18–20] or organic anions, such as malate [21], whereas the uptake of the osmotically important K^+ ion occurs *via* a K^+/H^+ exchange mechanism [18, 21, 22]. Furthermore, in some cases the active H^+ transport is responsible for the uptake of sugars, metabolites, and natural products [23], thereby increasing the osmotic potential of the cell sap.

Fig. 1 depicts the ATP-driven uptake of H^+ ions into tonoplast vesicles of coleoptiles of *Zea mays*.

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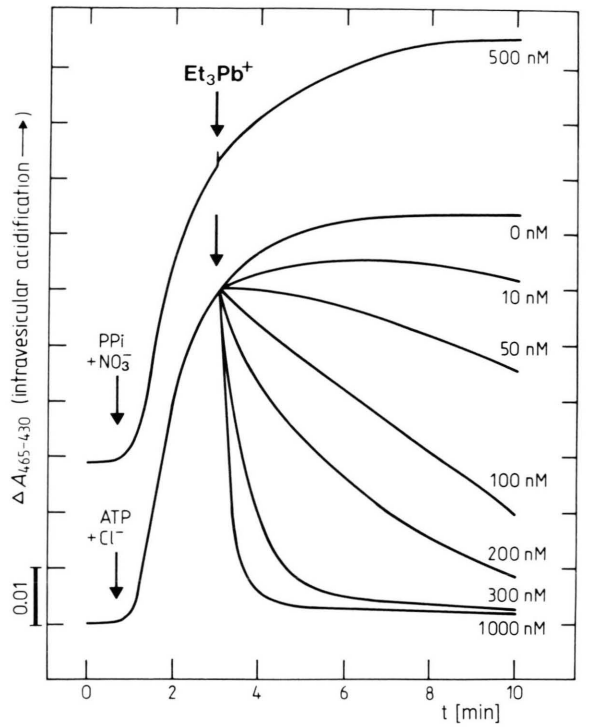

Fig. 1. Inhibition by Et_3Pb^+ of the ATP-driven intravesicular acidification of tonoplast vesicles from coleoptiles of *Zea mays* in the presence of 50 mM KCl. In the presence of NO_3^- (instead of Cl^-) the pyrophosphate(PPi)-driven acidification (which is NO_3^- insensitive in contrast to the H^+-ATPase [18]) is not abolished by Et_3Pb^+.

Under the given experimental conditions Cl^- is cotransported with the H^+ [18, 19]. Addition of Et_3Pb^+ at various concentrations caused an immediate destruction of the H^+ gradient. Even in the low concentration range of 10 nM the toxin stopped the ATP-dependent accumulation of protons immediately and a decrease of the H^+ concentration was initiated. A prerequisite for this drastic effect of Et_3Pb^+ is the presence of Cl^-. As shown in Fig. 2 the intravesicular acidification occurring in the presence of the anion fumarate was not inhibited by Et_3Pb^+. Addition of Cl^- at the 3rd minute increases the H^+ transport rate in the absence of Et_3Pb^+. In its presence, however, Cl^- induced a decrease of the H^+ concentration within the vesicles. This effect can best be explained by the assumption that the Et_3Pb^+ cation solubilized within the membrane is acting as a powerful Cl^-/OH^- exchanger (Fig. 5). The disappearence of accumulated protons can only occur if Cl^- ions transported into the vesicles are exchanged by OH^- from the medium, neutralizing the protons within the vesicles. Organic acids, such as fumarate, can not be exchanged for OH^- *via* Et_3Pb^+ (Fig. 2). A further indication for Cl^-/OH^- antiporter properties of Et_3Pb^+ is the fact that if Cl^- is substituted by NO_3^- the intravesicular acidification of tonoplast vesicles which is driven by the pyrophosphate (PPi)-dependent H^+-pump (insensitive to NO_3^- in contrast to the

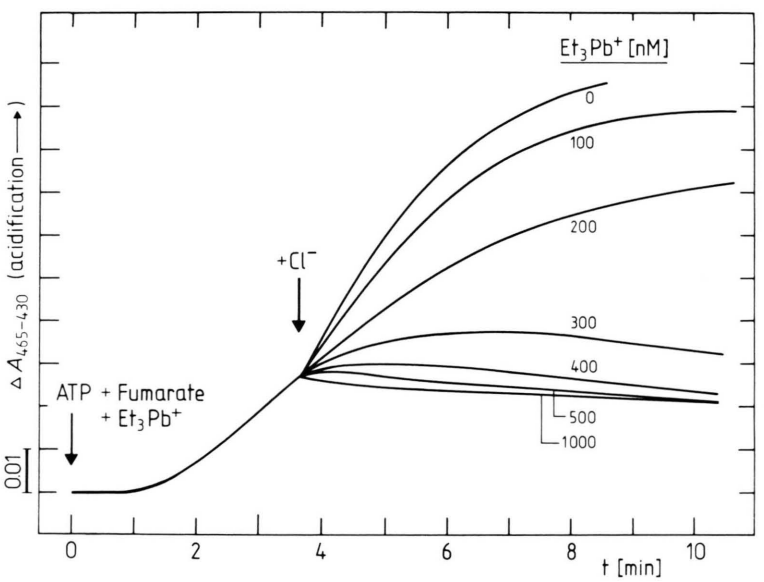

Fig. 2. ATP-driven intravesicular acidification of tonoplast vesicles in the presence of Et_3Pb^+. Initially fumarate was the anion, cotransported with the proton. Addition of Cl^- after the third minute enhances the H^+ uptake, but in the presence of Et_3Pb^+ Cl^- decreases the H^+ accumulation.

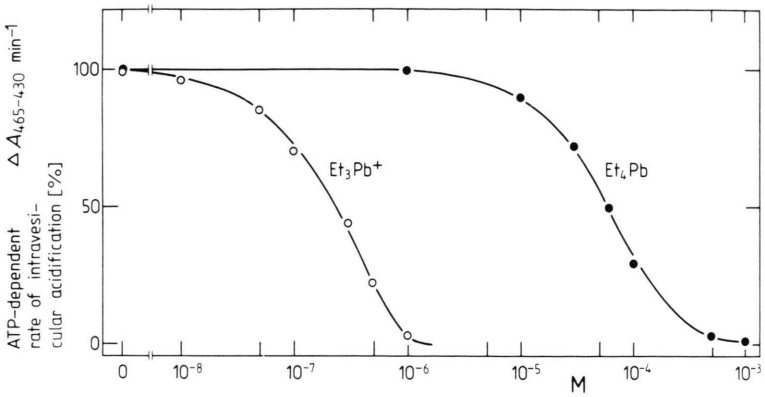

Fig. 3. Initial rate of the ATP-dependent intravesicular acidification of tonoplast-type vesicles in the presence of various concentrations of Et_3Pb^+ and Et_4Pb.

ATP-driven H^+-pump; see Fig. 5) could not be inhibited by the toxin (Fig. 1).

The functioning of Et_3Pb^+ as Cl^-/OH^- antiporter corresponds with similar mechanisms reported for triethyl-, tripropyl- or triphenyltin [24, 25].

It should be mentioned that in concentrations higher than 1 μM an additional inhibitory effect of Et_3Pb^+ was observed. The tonoplast-type H^+-pump activity depends on regulatory thiol groups on the enzyme [21]. SH-blocking agents, such as *p*-hydroxymercuribenzoate, or an oxidation of these sulfhydryl groups to disulfides, *e.g.*, by blue light or by H_2O_2, inactivated the enzyme reversibly; a rereduction by GSH restores the activity [21, 26]. Et_3Pb^+ interacts with these thiols of the H^+-ATPase at concentrations comparable with those employed for the inhibition of microtubule assembly [4]. But this SH-blocking effect might not be of importance under *in vivo* conditions because Et_3Pb^+, acting as Cl^-/OH^- exchanger, already disturbs cell metabolism in a much lower, nano-molar concentration range.

A comparison of the effects of Et_3Pb^+ and Et_4Pb on the ATP-dependent rates of the acidification of tonoplast vesicles (Fig. 3) shows that the oxidized charged molecule is 1000-fold more effective in abolishing the proton accumulation within tonoplast vesicles than Et_4Pb. The relatively small inhibitory effect of Et_4Pb may probably be caused by contamination with Et_3Pb^+ molecules, which are permanently formed in small amounts by oxidation from Et_4Pb.

The strong inhibitory effect of Et_3Pb^+ on the accumulation of ions within vacuolar vesicles should result in an immediate collapse of turgor of the intact cell. However, in experiments with coleoptiles auxin-induced elongation growth, which depends on a sufficient osmolarity of the cell sap, was inhibited only slowly and at higher concentrations of Et_3Pb^+ only (Fig. 4). This retarded effect of the toxin could be due to absorption (cuticle; cell wall) and, consequently, a poor penetration into the cytoplasm.

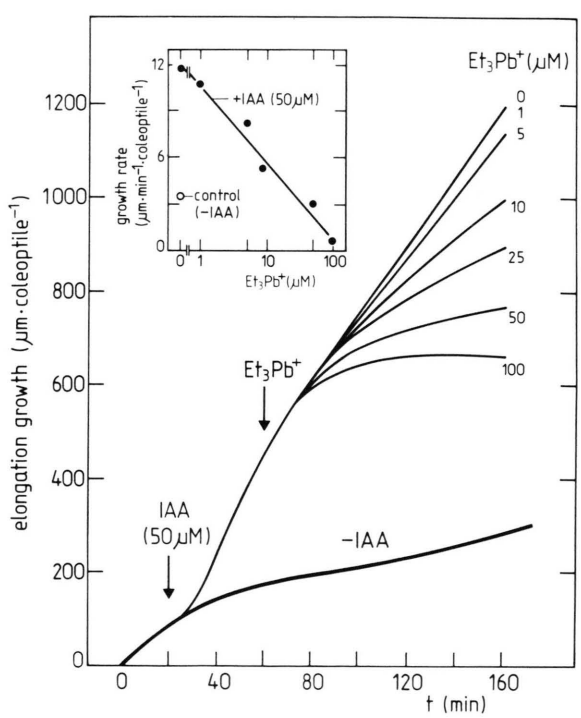

Fig. 4. Inhibition of auxin(IAA)-induced elongation growth of *Avena* coleoptile segments (1 cm in length) by various concentrations of Et_3Pb^+. Insert: Rate of elongation growth of coleoptile segments 3 h after addition of IAA (50 μM) and Et_3Pb^+ (various concentrations). IAA = Indole-3-acetic acid. Method see [28].

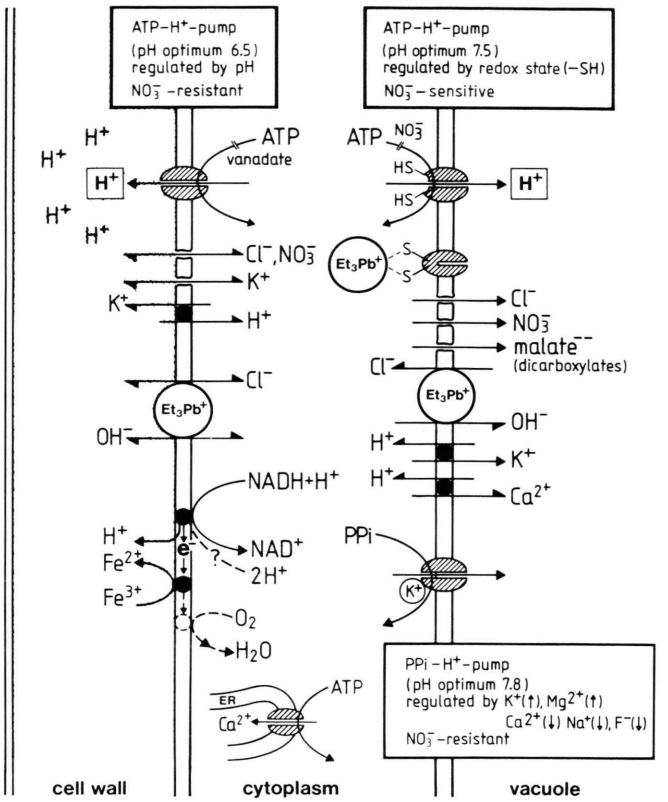

Fig. 5. Schematic presentation of the primary and secondary energized ion transport mechanisms in a plant cell as demonstrated in [18] and other recent publications [21, 26–29; 14, 30], and the sites of Et_3Pb^+ action as Cl^-/OH^- antiporter and SH-blocker, effective in nmolar and μmolar concentrations, respectively.

Therefore, the disappearence of the toxin from a solution containing fresh needles of conifers [10] can not give evidence to what degree cellular processes will be inhibited.

The effects of Et_3Pb^+ on plant cells by acting as an anion antiporter and a thiol blocker of the tonoplast-type H^+-pump are summarized in Fig. 5. The experimental basis of this scheme is provided in some recent publications [18 and 15, 19, 21, 26–29; 14, 30].

Acknowledgements

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