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Single and combined effects of Cd and Pb on the growth, medium pH, membrane potential and metal contents in maize (*Zea mays* L.) coleoptile segments

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ABSTRACT

The mechanisms of the toxic effects of Cd and Pb on plant cell growth are still poorly understood. In particular, little is known about their interactive effects, which usually occur in the environment. Moreover, the data that do exist in the literature are controversial. This study describes experiments that were performed with maize (*Zea mays*) coleoptile segments, which is a classical model system for studies of plant cell elongation growth. Cadmium and lead, which were added at 0.1 mM, reduced the endogenous and IAA-induced elongation growth of maize coleoptile cells. When both metals were added together or in sequence, their effect on IAA-induced growth was more toxic. The medium pH changes, which were measured simultaneously with growth, indicated that while Pb stopped IAA-induced proton extrusion, Cd only partially diminished it. Although Cd was generally more accumulated than Pb in the maize coleoptile segments, when IAA was added together with Pb, it significantly increased the accumulation of the metal. The short-term electrophysiological experiments showed that the addition of Cd caused the depolarisation of the membrane potential (E_m), whereas Pb caused membrane hyperpolarisation. In the long-term electrophysiological experiments, it was found that the Cd-induced E_m changes are complex. In conclusion, these results suggest that the effects of Cd and Pb as well as their combination on the elongation growth of maize coleoptile cells and the accumulation of the metals result, among others, from different ionic mechanisms by which each metal change the membrane potential of the cells.

1. Introduction

Natural processes and human activities are main causes of heavy metal contamination. Among the heavy metals, cadmium and lead are elements that are studied very often due to their toxicity. Both metals induce multiple direct and indirect effects on plants, resulting in reduced plant growth (reviewed in Nagajyoti et al., 2010; Asati et al., 2016; Hasan et al., 2017; Mishra et al., 2017; Singh et al., 2016). Among the effects that are caused by Cd and Pb, changes in the structure and function of the plasma membrane, which constitutes the first "intelligent" barrier for the entry of toxic ions into a cell, are crucial for growth and development. Alteration in the plasma membrane properties are manifested, e.g. in changes in the plasma membrane H^+-ATPase activity. This enzyme generates the gradient of the electrochemical potential of H^+, which is the driving force for the uptake and efflux of ions and metabolites across the plasma membrane. It should also be pointed out that changes in the plasma membrane electrogenic H^+-ATPase activity, which constitute the active component of the plasma membrane potential, also modulate the driving forces for the diffusion of all of the ions that cross the membrane. H^+-ATPase activity is also important for the regulation of cell turgor, which drives cell elongation. According to the so-called "acid growth hypothesis", auxin activates the PM H^+-ATPase, which acidifies the apoplasm and causes the activation of the enzymes that are involved in cell wall loosening (for a review see Hager, 2003; Rayle and Cleland, 1992). Activation of PM H^+-ATPase by auxin also induces the hyperpolarisation of the membrane potential, which causes the activation of the voltage-dependent, inwardly rectifying K^+ channels, the activity of which contributes to the water uptake that is necessary for cell expansion (reviewed in Hager, 2003; Kutschera, 1994; Kutschera and Wang, 2016).

It has previously been shown that the activity of PM H^+-ATPase was depressed in the presence of Cd (Astolfi et al., 2003; Fodor et al., 1995; Janicka-Russak et al., 2008; Kennedy and Gonsalves, 1989; Lindberg and Wingstrand, 1985; Obata et al., 1996; Ros et al., 1992). In turn, it is well known that the decrease in PM H^+-ATPase activity causes plasma membrane depolarisation. Such Cd-induced depolarisation of the plasma membrane has been found by numerous authors (Fiala et al., 2015a, 2015b; Karcz and Kurtyka, 2007; Kennedy and Gonsalves, 1987;

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Kurtyka et al., 2011b; Llamas et al., 2000; Pavlovkin et al., 2006; Sanz et al., 2009) and it has been shown that it depends on length of the exposure of cells to Cd, the concentration of the metal and the plant species.

Significantly less is known about the effect of Pb on H^+-ATPase activity and the membrane potential changes that are caused by this metal. It has previously been shown that Pb inhibits H^+-ATPase activity in *Zea mays* roots (Kennedy and Gonsalves, 1989; Tu and Brouillette, 1987) and that pre-treatment of wheat roots with vanadate, a H^+-ATPase inhibitor, significantly decreases the Pb content in wheat roots (Wang et al., 2007). The published data concerning the impact of Pb on the membrane potential of plant cells are scarce and often controversial. For example, in *Nitellopsis obtusa* cells, Pb at 0.1 mM caused the hyperpolarisation of the membrane potential, whereas at 1.0 mM it caused membrane depolarisation (Kurtyka et al., 2011b). The hyperpolarisation of the membrane potential in the presence of lead acetate at 5 mM was observed by Morse and Spanswick (1984) in the cells of *Nitella translucens*. In turn, the depolarisation of the membrane potential at 0.1 mM of Pb was found in *Zea mays* roots by Kennedy and Gonsalves (1987).

In the environment, Pb and Cd are usually present together, thus causing a combined effect that may differ significantly from the one that is induced by each metal separately. Despite the abundant literature on single Cd or Pb exposure, only a few studies have been performed in which the combined effect of both metals, were determined (An et al., 2004; Lanier et al., 2016; Panich-Pat et al., 2010; Srivastava et al., 2014).

In this study, we chose the IAA-induced growth of maize coleoptile segments (a classical model system for studies on the elongation growth of plant cells in which the number of cells is constant and the organ grows only by elongation, see Kutschera and Wang, 2016) as the main parameter in order to assess Pb and Cd toxicity. In addition, medium pH, the membrane potential and the accumulation of metals were also determined. The phytohormone auxin (indole-3-acetic acid; IAA) is a major hormone that regulates many processes in plants, including cell elongation, cell division and responses to abiotic stresses (recently reviewed in Ma et al., 2018). Among these stresses faced by plants, the interaction between auxin and heavy metal toxicity is of particular interest for plant productivity (reviewed in Bücker-Neto et al., 2017). It should be also added that most of crucial evidence on the mechanism of auxin action in plant cell growth was obtained from maize coleoptile segments (recently reviewed in Arsuffi and Braybrook, 2018).

The main goal of our experiments was to study the toxic mechanisms by which Cd and Pb diminish plant growth. This goal was realised by comparing the single and combined effects of Cd and Pb on: (1) the endogenous and IAA-induced growth of maize coleoptile segments, (2) the membrane potential (E_m) of parenchymal coleoptile cells that were treated with IAA or with no IAA and (3) the accumulation of both metals in maize coleoptile segments that were incubated in the presence and absence of IAA. In some cases, the effects of Cd and Pb on the medium pH changes, which were measured simultaneously with growth, were also determined. This experimental design can provide new data on the toxic effects of Cd and Pb on plant growth.

2. Materials and methods

2.1. Plant material

Caryopses of maize (*Zea mays* L. cv. Cosmo) were soaked in tap water for 2 h, sown on wet lignin in plastic boxes, and placed in a growth chamber (Type MIR-533, Sanyo Electric Co., Japan) at 27 ± 1 °C. The experiments were carried out with 10 mm-long coleoptile segments that were cut from four-day-old etiolated maize seedlings. Coleoptile segments with the first leaves removed were excised 3 mm below the tip and collected in an incubation medium of the following composition (control medium): 1 mM KCl, 0.1 mM NaCl, 0.1 mM $CaCl_2$, initial pH 5.8–6.0. Conditions for growing the maize seedlings were previously described by Karcz and Burdach (2002) and Burdach et al. (2014).

2.2. Chemicals

An aqueous stock solution (1 mM) of indole-3-acetic acid (IAA) (Serva, Heidelberg, Germany) was prepared with the potassium salt of IAA. IAA at a final concentration of 10 μM was used. Cadmium ($CdCl_2 \times 2.5\ H_2O$) (Fluka, Switzerland) and lead ($PbCl_2$) (Fluka, Switzerland) were dissolved in deionised water and used at a final concentration of 0.1 mM.

The concentration of both metals (0.1 mM) was selected in accordance with our previous studies (Karcz and Kurtyka, 2007; Kurtyka et al., 2011c; Małkowski et al., 2005) as well as by those in experiments that were performed by other authors (Janicka-Russak, 2008; Kennedy and Gonsalves, 1987; Llamas et al., 2000; Pavlovkin et al., 2006; Puertas-Mejía et al., 2010; Sanz et al., 2009; Wójcik and Tukiendorf, 2005), who showed that Cd and Pb at 0.1 mM caused a moderate toxic effect on plant growth.

2.3. Growth and pH measurements

Growth experiments and the pH of the incubation medium from the same tissue sample were performed in an apparatus that permitted the simultaneous measurement of both parameters (Burdach et al., 2014; Polak et al., 2012). pH measurements were carried out using a pH meter (Type CI-316; Elmetron, Poland) and a pH electrode (OSH 10–10; Metron, Poland). Growth experiments were carried out using an angular position transducer (TWK Electronic, Düsseldorf, Germany), which permitted the high resolution measurements of growth rate (Burdach et al., 2014; Polak et al., 2012). In the experiments, 60 coleoptile segments were arranged vertically in three narrow glass pipettes (20 segments in each), which were connected by a silicone hose. Coleoptile segments were incubated in an intensively aerated medium of the following composition (control medium): 1 mM KCl, 0.1 mM NaCl, 0.1 mM $CaCl_2$ and initial pH 5.8–6.0 (Kurtyka et al., 2011a). The volume of the incubation medium in the growth and pH-measuring apparatus was 18 ml (0.3 ml segment^{-1}). As was described previously by Karcz et al. (1995), the incubation medium in this system also flows through the lumen of the coleoptile cylinders. This feature permitted the experimental solutions to be in direct contact with the interior of the segments, which significantly enhanced the elongation growth of the coleoptile segments and proton extrusion. The circulation of the medium was driven by a peristaltic pump (Type Peri-Star PRO, World Precision Instruments Inc., USA). All manipulations and growth experiments were conducted under dim green light. The temperature of all of the solutions in the elongation measuring system was thermostatically controlled at 25 ± 0.5 °C. The length of the coleoptile segments was sampled every 3 min using a CX721 converter (Elmetron, Poland). The growth rate was expressed in μm s^{-1} cm^{-1}.

2.4. Electrophysiology

The electrophysiological experiments were performed on ten-mm-long coleoptile segments that were prepared in the same manner as those for the growth experiments. A standard electrophysiological technique was used for the membrane potential measurements, as was previously described by Karcz and Burdach (2002) and Burdach et al. (2014). The membrane potential (E_m) was measured by recording the voltage between a 3 M KCl-filled glass micropipette that was inserted into the parenchymal cells and a reference electrode in the bathing medium that contained the same composition as that used in the growth experiments. Prior to the electrophysiological experiments, the coleoptile segments were preincubated for 1 h in an aerated bathing medium. After this period, one of them was transferred into a perfusion