BIOLOGIJA. 2005. Nr. 1. P. 31–34

Al^{3+} induced membrane potential changes in *Nitellopsis obtusa* cells

V. Kisnierienë*,
V. Sakalauskas

Vilnius University, Department of Biochemistry and Biophysics, M. K. Èiurlionio 21, LT-03101 Vilnius, Lithuania

To study the mechanism of aluminium toxicity in plant cells, the effects of aluminium on the electrogenesis of plasma membrane were investigated. We analyzed Al^{3+} induced membrane potential (E_m) changes and potential changes during generation of the action potential (AP) in the internodal cells of *Nitellopsis obtusa*. Al^{3+} modified E_m decrease significantly. Al^{3+} didn't attenuate the amplitude of the action potential too much, though the peak value of the action potential increased. The shift of the second stage of AP repolarization was about 32 mV in positive direction as a result of Al^{3+} treatment. Al^{3+} has been suggested to affect Ca^{2+} homeostasis and H^+-ATPase activity.

Key words: aluminium (Al toxicity), *Characeae, Nitellopsis obtusa*, membrane potential, action potential, Ca^{2+} homeostasis, electrogenic H^+ pump

INTRODUCTION

Aluminium is the third most abundant chemical element in the Earth's crust. Aluminium toxicity and long-term accumulation in humans has been blamed as a cause of osteoporosis and Alzheimer's disease, and in the wider environment the release of aluminium from soil by acid rains has seriously affected forests and aquatic life. The most important symptom of Al toxicity in plants is the inhibition of root growth. Aluminium toxicity is no longer a question, although the mechanisms by which this toxicity is exerted have not been fully established. Al could have diverse effects and acts differently on different species. Part of the difficulty of studying Al-related processes in plants can be attributed to the complex chemistry of A1 [1, 2]. A1 hydrolyzes in solution so that the trivalent A1 types, $A1^{3+}$, dominate in acid conditions (pH < 5), whereas the $Al(OH)^{2+}$ and $Al(OH)^+$ species form with the pH increase. At near-neutra1 pH the solid phase $Al(OH)_3$ or gibbsite occurs, whereas $Al(OH)_4^-$, or aluminate, dominates in alkaline conditions. Many of these monomeric A1 cations bind to various organic and inorganic ligands, organic acids, proteins, and lipids. Al toxicity can emerge due to the effect of Al ions on cell walls as well as the toxic effect of Al ions on the plasma membrane of younger outer cells in roots or on the root symplasm. The root zone-specific depolarization of the plasma membrane surface potential in squash has been shown to be an early symptom of Al toxicity [3]. Sivaguru et al. [4] found that Al caused instantaneous plasma membrane depolarization in root cells of an Al-sensitive maize cultivar, and the intensity of depolarization varied with the root growth zone. Numerous biochemical and physiological processes have been demonstrated to be affected within a period from minutes to hours of exposure to Al, such as disturbance of cytoplasmic Ca^{2+} and pH homeostasis [5–12] inhibition of H^+-ATPase activity in the plasma membrane of the root tip cells [3], changes in membrane surface charge [13, 14], inhibition of cation uptake [15–17] and inhibition of plasmodesmata-mediated intercellular transport [18]. Takabatake & Shimmen [19] reported that electrogenesis at the membrane was inhibited by treatment with $AlCl_3$; they used internodal cells of *Chara corallina*. Taylor et al. [20] provided the direct measurement of Al transport across the plasma membrane and tonoplast in single cells. *Characean* cells have proven to be the most suitable material for analyzing the electrical characteristics of membranes of plant cells [21]. In this paper, we report aluminum-induced membrane potential changes in *Nitellopsis obtusa*, another *Characean*.

*Author for correspondence. E-mail: vilma.kisnieriene@gf.vu.lt

MATERIALS AND METHODS

Internodal cells of freshwater stonewort *Nitellopsis obtusa* (*Devs.*) *J. Gr.* (*Characeae*) were used throughout the experiment. The cells were originally harvested by kedge anchor from a depth of about 3–7 m from Lake Stanka near Vilnius. The plants were transported to the laboratory in plastic bags filled with lake water. In laboratory the alga was cul-

tured in a glass aquarium containing tap water at room temperature (18–23 °C) under illumination with cool white fluorescent lamps (12–14 h/day). The light intensity was about 3 $W \cdot m^{-2}$ at the surface of water. Before the experiments internodal cells were isolated from neighboring cells and branchlets. The internodes (each cell length ranging from 3 to 5 cm) were kept at least overnight in artificial pond water (APW) containing 0.1 mM KCl, 1.0 mM NaCl, 0.1 mM $CaCl_2$, (pH 5.6). APW was used as the basic solution. Aluminium treatment was caried out by incubating internodal cells in this basic APW supplemented with 1 mM $AlCl_3$. Addition of 1mM $AlCl_3$ caused a decrease in pH of APW from 5.6 to about 4. During experiments the cells were placed in a plexiglass chamber and continuously bathed in a flowing solution of APW or test solution APW + $AlCl_3$ at a rate of about 1 $ml \cdot min^{-1}$.

Transmembrane potentials were measured by a standard microelectrode technique [22]. The microelectrodes had a tip diameter of <1 μm and were made of borosilicate glass capillaries (Kwik-Fil™, World Precision Instruments, Inc., USA). Reference electrodes were filled with 3M KCl in agar-agar jelly and immersed in the experimental solution. A microelectrode was inserted into the cell to measure the electrical properties of the PM. Membrane potentials were measured 1 h after insertion, during which cytoplasmic streaming recovered its normal rate. It had been stopped by mechanical shock while inserting the microelectrode. Action potentials were elicited by injecting a depolarizing current between two pools using Ag / AgCl wires. Data were A / D converted (16 bits, ADS7805P, Burr-Brown Corporation) and stored in the computer memory for a further analysis of membrane parameters. A schematic diagram of the electrical apparatus and electrode arrangement used is shown in Fig.1.

Standard errors are given for means n = 6. All statements on statistical significance are based on a confidence level of 95↑. The Statistica 6.0 (StatSoft) software was used for statistical analysis.

RESULTS AND DISCUSSION

Characeae are one of the most suitable objects for studying the electrophysiology of plant cells [21]. In higher plants, it is difficult to isolate the electrical signal of a target cell from those of surrounding cells. By taking advantage of this material, we analyzed the effects of aluminium (Al^{3+}) on electrogenesis at the plasma membrane of internodal cells of *Nitellopsis obtusa* – the membrane potential (E_m) and the potential changes during generation of the action potential (AP).

The plasma membrane is a barrier to the passive movement of ions into plant cells, and some responses of plant cells to Al are related to the alteration of plasma membrane properties. It has been proposed that the plasma membrane depolarization itself may be an inhibitory stress.

In our experiments, before treatment with Al^{3+}, E_m was –238 ± 8.6 mV. After one hour of treatment, the cell membrane depolarised to –217 ± 7.1 mV. Al^{3+} determined the E_m decrease by 21 mV. After removal of Al^{3+} from the bathing solution the effect of Al^{3+} was reversible. As Takabatake and Shimmen [19] have reported, the cell membrane of *Chara corallina* depolarised from –197 mV to –104 mV (93 mV) after 1 h of incubation in Al^{3+} medium. Reid et al. [17] have shown that the cell membrane of *Chara* was slightly hyperpolarized by addition of Al^{3+} to the external medium after 30 min. These results are inconsistent with our results. Al may have diverse effects and act differently in different species. The resting membrane potential of *Characean* cells is composed of two components – the passive difusion potential and the active potential generated by an electrogenic proton pump. The second component is more important in *Nittelopsis obtusa* than in other *Characeans*,

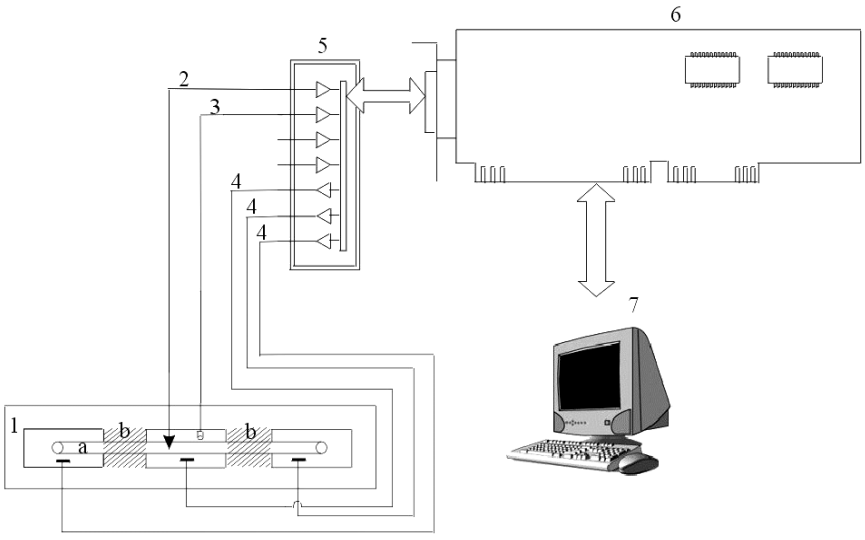

Fig. 1. Shematic diagram of experimental set-up
1. Pexiglass chamber with three compartments: *a* – internodal *Nitellopsis obtusa* cell, *b* – vaseline gap (isolation); 2. Glass microelectrode; 3. Reference electrode; 4. External Ag / AgCl current electrodes; 5. Preamplifier and control block; 6. Universal computer I / O and data acquisition system; 7. Personal computer

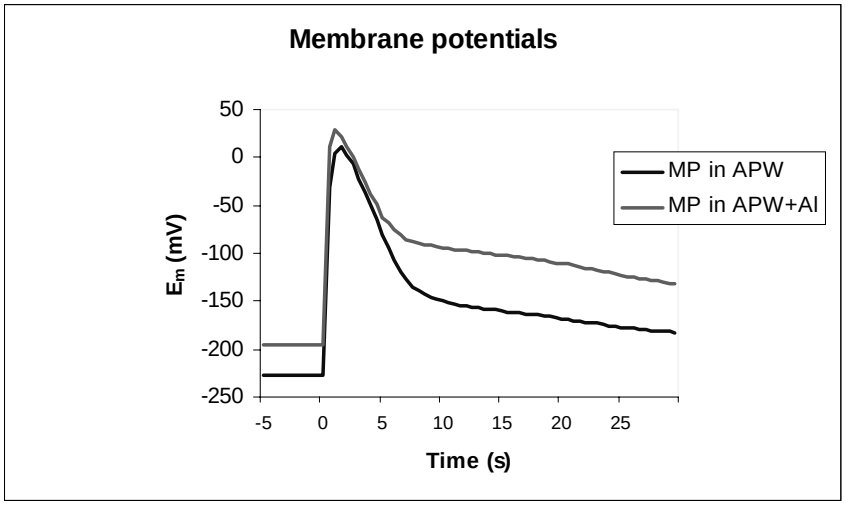

Fig. 2. Typical example of the effect of Al on *Nitellopsis obtusa* membrane and action potential (in APW and APW + Al^{3+} respectively)

and we assume that Al influences H^+-ATPase activity. The modulation of H^+-ATPase activity, and thus H^+ pumping, is involved in a wide range of fundamental cellular processes, such as the formation and maintenance of an electrochemical gradient that serves as a driving force for the secondary ion transport. Since ATPase activity may play an important role in generating the plasma membrane potential, the toxic effects of Al on ATPase system could be reflected by changes in membrane potential [23].

The effect of Al^{3+} on the generation of the action potential (AP) was examined by comparing the shape, amplitudes and peak value of AP before and after treatment with Al^{3+}. The shape of the action potential remained substantialy unaffected (see Fig. 2). Al^{3+} didn't attenuate the amplitude of the action potential significantly, though the action potential peak value increased by 13.84 mV. The AP peak values were +1.33 ± 3.81 mV and +15.17 ± 2.03 mV in APW and APW+Al^{3+}, respectively. The second stage of repolarization during AP started at –127.83 ± 3.32 mV in the control solution and –95.67 ± 4.66 mV in the test solution (the shift was about 32 mV). The parameters are expressed as mean ± standard error. Table 1 summarizes the effect of Al on the membrane potential (E_m) and potential changes during the generation of the action potential (AP).

The action potentials involve a transient influx of Ca^{2+} to the cytoplasm, effluxe of $K^+$and Cl^-. The depolarization phase of AP is caused by an influx of Cl^-. AP repolarization is comprised of two stages. The repolarization phase is due to the outward K^+ flow and the activity of the electrogenic pump at the plasma membrane. The second stage of repolarization during AP is supposed to be related to the operation of the electrogenic H^+-pump in the excitable membrane. The K^+ efflux is controlled by calcium-dependent potassium channels, which close when the Ca^{2+}_{cyt} decreases [10]. Classic experiments showed that the action potential peak strongly depended on the external concentration of Ca^{2+}. The increased Ca^{2+}_{cyt} acts as a signal to elicit changes in a series of biochemical and physiological processes. The idea that Al could disturb cellular metabolism by disrupting Ca^{2+} homeostasis was developed from the known antagonism between Al and Ca^{2+}. Al might distrupt Ca^{2+}-dependent metabolism by maintaining a higher than normal Ca^{2+} level in the cytoplasm. Disruption of cytoplasmic Ca^{2+} homeostasis has been suggested as a primary trigger of Al toxicity [24]. It is still controversial whether Ca^{2+}_{cyt} originates from the external medium or from internal stores such as endomembranes. The source of Ca^{2+} for increasing cytosolic Ca^{2+} during the Al stress is both extracellular (Al depolarizes the plasma membrane, thus potentially enhancing the flux of Ca^{2+} through depolarization-activated channels that are only partially inhibited by Al in the absence of membrane depolarization) and intracellular (increased Ca^{2+}_{cyt} sti-

Table. **Effect of Al^{3+} on membrane parameters**

Cell number	Membrane potential, mV		Peak value of AP, mV		Second stage of repolarization, mV	
	APW	APW + Al^{3+}	APW	APW + Al^{3+}	APW	APW + Al^{3+}
1	–243	–212	–10	14	–132	–89
2	–227	–194	7	14	–130	–95
3	–196	–160	–8	8	–118	–96
4	–254	–230	11	21	–133	–99
5	–222	–208	2	15	–119	–82
6	–254	–236	6	19	–135	–113
Mean ± SE	–232.7 ± 10.1	–206.7 ± 12.3	1.3 ± 3.84	15.2 ± 2.1	–127.8 ± 3.3	–95,7 ± 4,7

mulates Ca^{2+}-release channels in the tonoplast and the endoplasmic reticulum). Depolarization causes elevation of the second mesenger IP3 and a consequent mobilization of Ca^{2+} from internal stores [25].

The present study has shown aluminium to affect the plasma membrane properties. The results presented above are consistent with the idea that Al may disrupt Ca^{2+} homeostasis. Analysis of the action potential may validate Al effect on H^+-ATPase.

Received 6 January 2005
Accepted 24 February 2005

References

1. Delhaize E, Ryan PR. Plant Physiology 1995; 107: 315–21.
2. Kinraide TB. Plant Soil 1991; 134: 167–78.
3. Ahn SJ, Sivaguru M, Chung GC, Rengel Z, Matsumoto H. J Exper Bot 2002; 53: 1959–66.
4. Sivaguru M, Baluska F, Volkmann D, Felle HH, Horst WJ. Plant Physiol 1999a; 119: 1073–82.
5. Lindberg S, Strid H. Physiol Plant 1997; 99: 405–14.
6. Jones DL, Gilroy S, Larsen PB, Howell SH, Kochian LV. Planta 1998; 206: 378–87.
7. Jones DL, Kochian LV, Gilroy S. Plant Physiol 1998; 116: 81–9.
8. Zhang WH, Rengel Z, Kuo J, Plant Journal 1998; 15: 147–51.
9. Zhang WH, Rengel Z, Kuo J, Yan G. Annals of Botany 1999; 84: 559–64.
10. Plieth C, Sattelmacher B, Hansen UP. Knight MR. Plant Journal 1999; 18: 643–50.
11. Zhang WH, Rengel Z. Austral J Plant Physiol 1999; 26: 401–9.
12. Ma QF, Rengel Z, Kuo J. Ann Bot 2002; 89: 241–4.
13. Kinraide TB, Ryan PR, Kochian LV. Plant Physiol 1992; 99: 1461–8.
14. Kinraide TB, Ryan PR, Kochian LV. Planta 1994; 192: 104–9.
15. Rengel Z, Robinson DL. Agron J 1989; 81: 208–15.
16. Huang JW, Shaff JE, Grunes DL, Kochian LV. Plant Physiol 1992; 98: 230–7.
17. Reid RJ, Tester MA, Smith FA. Planta 1995; 195: 362–8.
18. Sivaguru M, Fujiwara T, Samaj J, Baluska F, Yang ZM, Osawa H, Maeda T, Mori T, Volkmann D, Matsumoto H. Plant Physiol 2000; 124: 991–1005.
19. Takabatake R, Shimmen T. Plant Cell Physiol 1997; 38(11): 1264–71.
20. Taylor GJ, McDonald-Stephens JL, Hunter DB, Bertsch PM, Elmore D, Rengel Z, Reid RJ. Plant Physiol 2000; 123: 987–96.
21. Shimmen T, Mimura T, Kikuyama M, Tazava M. Cell Stuct Funct 1994; 19: 263–78.
22. Purves RD. Microelectrode Methods for Intracellular Recording and Ionophoresis. Academic Press 1981: 208.
23. Lindberg S, Szynkier K, Greger M. Physiol Plant 1991; 83: 54–62.
24. Rengel Z, Zhang WH. New Phytologist 2003; 159: 295–314.
25. Wacke M, Thiel G. J Gen Physiology 2001; 118: 11–21.

V. Kisnierienë, V. Sakalauskas

ALIUMINIO JONØ POVEIKIS MENTURDUMBLIO *NITELLOPSIS OBTUSA* LÀSTELIØ MEMBRANINIAMS POTENCIALAMS

Santrauka

Buvo tirta menturdumblio *Nitellopsis obtusa* làsteliø plazminës membranos elektrogenezë, paveikus jas Al^{3+} jonais. Nustatytos membraninio potencialo ir veikimo potencialø maksimalios amplitudës vertës ir repoliarizacijos antros fazës pradþios priklausomybë nuo aliuminio jonø. Árodyta, kad veikiant Al^{3+} jonams, membraninis potencialas statistiðkai patikimai sumaþëja. Veikimo potencialo maksimali amplitudës vertë reikðmingai padidëjo, o antroji repoliarizacijos stadija prasidëjo 32 mV aukðèiau. Remdamiesi gautais duomenimis galime daryti prielaidà, kad Al^{3+} veikia Ca^{2+} homeostazæ ir H^+-ATPasës aktyvumà.