Short communication

Ba^{2+} Ions Hyperpolarize Algal Cell Membrane and Enhance Plasmalemma Resistance without Affecting Ion and Water Contents

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Abstract. Culturing the fresh-water alga *Hydrodictyon reticulatum* for 9 days in the presence of 0.2 and 0.4 mmol/l Ba^{2+} increases the membrane potential about twice and the plasmalemma resistance 5–10 times without having any significant effect on the cell contents of water and Na^+, K^+ and Cl^- ions. While in control cells the membrane potential is determined by the diffusion potential of K^+, in Ba^{2+}-treated cells the factor determining membrane potential and resistance is the unequal distribution of protons across the membrane.

Key words: Barium ions — Algal cell membrane — Electrical membrane characteristics — *Hydrodictyon reticulatum*

Fundamental questions concerning the ion relations in algae include the relationship between cell wall elasticity and turgor, osmoregulation and ion pumps. Our previous study of the fresh-water alga *Hydrodictyon reticulatum*, which does not use organic osmolytes for volume regulation, showed that by affecting the cell wall growth by low concentrations of different sugars it is possible to manipulate the turgor either without or with a parallel effect on intracellular sodium concentration (Janáček et al. 1995). The water and ion relations can be affected, apart from the sodium pump, also by the distribution of other ions, particularly potassium. Among substances known to affect potassium fluxes, Ba^{2+} ions, known to inhibit, e.g., the K^+ conductance of the *Chara corallina* plasmalemma (Tester 1988), had striking effects on the electrical properties of the *H. reticulatum* plasmalemma. These effects are the subject of this communication.

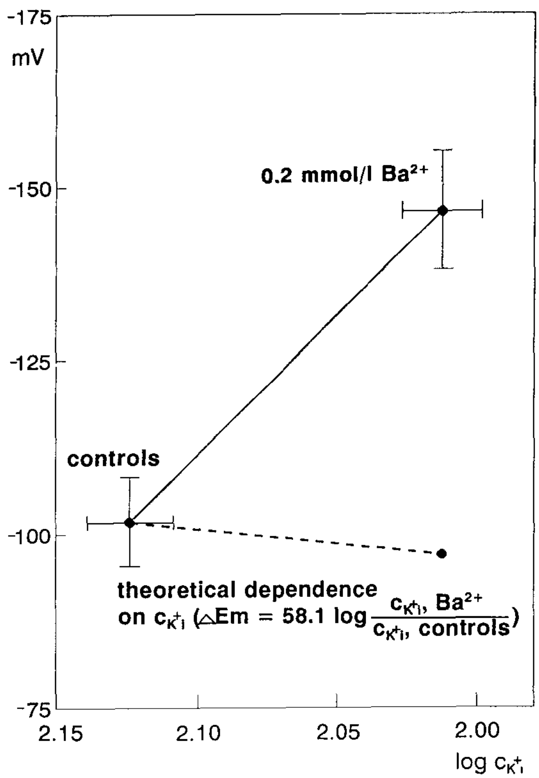
The alga *Hydrodictyon reticulatum* (L.) Lagerh. was cultured at room temperature under sterile conditions in artificial pond water with trace elements and leaf decoction as described elsewhere (Janáček et al. 1995). At the start of the experiment, small nets of algae were transferred into sterilized fresh culture medium without the soil decoction containing a specified concentration of $BaCl_2$, and were left to grow in the medium for 9 days. Subsequently, electrophysiological parameters of the cells (Metlička 1980) were measured using glass microelectrodes of up to 10 MOhm resistance and direct current pulses of about 10^{-8} A and 300 ms duration. Algal samples of about 50 mg fresh weight were collected after the growth period and their water content was determined from the weight loss after drying overnight at 95 °C in teflon vessels. The contents of sodium, potassium and chloride ions were determined after extracting the samples with 10 mmol/l H_2SO_4. Sodium and potassium were estimated using an EEL flame photometer, and potentiometric titration was used to determine the content of chloride ions. Concentrations were calculated by dividing ion contents by the corresponding water contents.

Table 1. Effect of growth in the presence of Ba^{2+} on membrane potential and resistance, water and ion contents in *H. reticulatum*

	E [mV]	R [Mohm]	H_2O [kg/kg DS]
Controls	-104.0 ± 7.4 $[n = 6]$	2.5 ± 0.3 $[n = 6]$	14.05 ± 0.50 $[n = 8]$
0.2 mmol/l Ba^{2+}	-178.2 ± 4.5 $[n = 6]$ $p \ll 0.001$	12.6 ± 0.7 $[n = 6]$ $p \ll 0.001$	13.09 ± 0.20 $[n = 8]$ n. s.
0.4 mmol/l Ba^{2+}	-245.5 ± 9.7 $[n = 6]$ $p \ll 0.001$	21.9 ± 1.3 $[n = 6]$ $p \ll 0.001$	12.14 ± 0.38 $[n = 8]$ $p < 0.01$
	$C_{K^+,i}$[mmol/l]	$C_{Cl^-,i}$[mmol/l]	$C_{Na^+,i}$[mmol/l]
Controls	117.1 ± 6.3 $[n = 8]$	82.7 ± 3.4 $[n = 8]$	1.27 ± 0.06 $[n = 8]$
0.2 mmol/l Ba^{2+}	115.8 ± 1.9 $[n = 8]$ n.s.	77.6 ± 4.2 $[n = 8]$ n.s.	1.19 ± 0.06 $[n = 8]$ n.s.
0.4 mmol/l Ba^{2+}	120.7 ± 3.8 $[n = 8]$ n.s.	80.2 ± 5.0 $[n = 8]$ n.s.	1.16 ± 0.05 $[n = 8]$ n.s.

As seen from Table 1, barium ions had no significant effects on the intracellular concentrations of the principal ions and exhibited but a marginal tendency to reduce the cell water content. On the other hand, they exerted an extremely

Figure 1. Intracellular potential of barium treated cells of the alga *Hydrodictyon reticulatum* is not related to the Nerst-Donnan potential of potassium ions.



significant effect on cell membrane potential and resistance. The resistance units are somewhat arbitrary, corresponding roughly to the whole cell surface, i.e. about 0.02 cm^2.

One observation appeared to be especially important: as shown in Figs. 1 and 2, quite unlike the control cells, in algae grown for 9 days in the presence of $BaCl_2$ the membrane potential across the plasmalemma bore no relationship to the Nernst-Donnan potential calculated from the distribution of potassium ions, obviously due to the blockage of ion conducting pathways in the membrane. The observed membrane potential and resistance must therefore be determined by ions different from potassium. Thick suspensions of illuminated algae are known to reach a pH of 10 by taking up bicarbonate anions in exchange for hydroxyl anions (Rybová et al. 1980), pH at the outer surface of the plasmalemma is bound to be still higher. Intracellular pH is kept near 6.0, as demonstrated by permeabilizing algal cells with nystatin (Rybová et al. 1980). A probable source generating the high membrane potential in barium-treated cells is therefore the unequal distribution of protons across the plasmalemma. To test this hypothesis, during the microelectrode measurement the original medium was replaced by the same medium acidified by

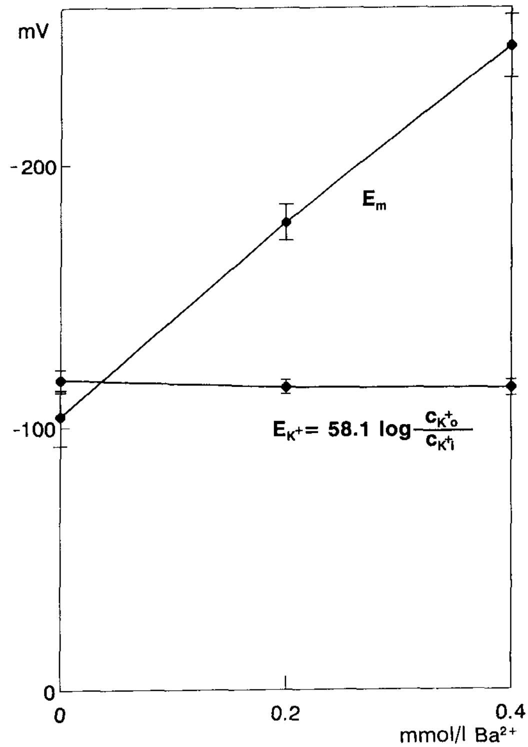


Figure 2. Barium ions increase the plasmalemma potential difference proportionally to their concentration. The potential is not determined by the distribution of potassium ions.

phosphoric acid from the original pH in the bulk 7.15 to pH 3.45. The only changes in the medium composition were therefore in phosphate concentration and in pH. Following this medium replacement, the intracellular potential of cells grown for 10 days in the presence of 0.4 mmol/l $BaCl_2$ dropped from −236 mV to −140 mV (a drop by 80 mV was recorded in another experiment). Even more striking was a 5-fold drop in plasmalemma resistance (mean of 2 experiments). This strongly indicates that in Ba^{2+}-grown cells protons are the principal conductor of the electric charge in the plasmalemma and determine the membrane potential according to the Hodgkin-Horowicz equation:

$$E = \frac{G_{K^+}E_{K^+} + G_{Na^+}E_{Na^+} + G_{Cl^-}E_{Cl^-} + G_{H^+}E_{H^+}}{G_{K^+} + G_{Na^+} + G_{Cl^-} + G_{H^+}}$$

(G are conductances, and E the Nernst-Donnan potentials for individual ionic species). We believe that in barium-treated cells $G_{H^+} \gg G_{K^+}, G_{Na^+}, G_{Cl^-}$.

In control algae not treated with barium ions the lowering of external pH to the value of 3.47 reduced the value of the intracellular potential, −118 mV, by 40 mV and increased the plasmalemma resistance from 2.2 to 4.6 MOhm, probably owing to a pharmacological effect of protons on the ionic channel conductance. We do not dare to interpret this effect in terms of the above equation without an exact knowledge of the pH value at the two surfaces of the plasmalemma. The effect was fully reversible.

Our results imply that in Ba^{2+}-treated cells of *Hydrodictyon reticulatum* the membrane potential is definitely not determined by the distribution of potassium ions. These results touch upon an old and important problem of whether K^+ is indeed the ion that nearly universally determines membrane potential in cells. Years ago, Grundfest and co-workers (Grundfest et al. 1954; Grundfest 1955) injected concentrated potassium salts into cells of squid giant axon and found no corresponding hyperpolarization. Our data indicate that when the role of the Nernst-Donnan potential of K^+ ions is minimized by blocking K^+-conductance pathways by Ba^{2+}, unequal distribution of protons becomes the factor determining the membrane potential in this alga.

Acknowledgements. The work was supported by grant No. 520101 of the Academy of Sciences of the Czech Republic.

References

Grundfest H. (1955): The nature of the electrochemical potentials of bioelectric tissues In: Electrochemistry in Biology and Medicine (Ed. T. Shedlovsky), p. 141, John Wiley & Sons, New York, London

Grundfest H., Kao C.-Y., Altamirano M. (1954): Bioelectric effects of ions microinjected into the giant axon of *Loligo* J. Gen. Physiol. **38,** 245—282

Janáček K., Nešpůrková L., Beneš I., Metlička, R , Sigler K. (1995): Modifications of algal cell wall properties and a turgor-dependent sodium pump by external carbohydrates. Comptes Rendus Ac. Sci. Paris/Life Sci. **318,** 13—16

Metlička R. (1980): Membrane resistance of the alga *Hydrodictyon reticulatum*. Z. Pflanzenphysiol. **96,** 18—193

Rybová R., Janáček K., Kleinová-Slavíková M. (1980): Light-dependent pH changes in suspensions of the alga *Hydrodictyon reticulatum*. Z. Pflanzenphysiol. **97,** 5—12

Tester M. (1988): Pharmacology of K^+ channels in the plasmalemma of the green alga *Chara corallina*. J. Membrane Biol. **103,** 159–169

Final version accepted April 18, 1996