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Effect of sodium humate on membrane potential in internodal cells of *Nitellopsis obtusa*

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Abstract

Membrane potential and resistance changes in *Nitellopsis obtusa* induced by Na-humate as a function of external pH were investigated. The administration of Na-humate at concentrations of 12.5, 25, 50 and 100 mg·dm^{-3} brought about a hyperpolarization of the membrane potential and a drop of d.c. resistance in the pH range between 4.0 and 9.0. Depolarization of the membrane potential induced by low pH was counteracted by Na-humate. The electrophysiological effects of Na-humate are compared with those of IAA.

Key words: humate, membrane potential.

INTRODUCTION

Paszewski et al. (1957) found that chromatographically purified fraction of humus referred to as hymetomelanic acids stimulates the growth of oat coleoptile segments in a way similar to auxin. However, no indolyl compound has been found in the humic fractions used in these experiments. Gumiński (1972) tried to explain the stimulatory action of humic substances as due to the exchange of cations between the root cells and the sorption complexes in the environmental medium. Kyć (1970) investigated the dependence of the sodium humate stimulatory action on the growth of a *Scenedesmus quadricauda* culture on the pH of the external medium. The author came to the conclusion that sodium humate relieves injurious influence of unsuitable pH values of external medium on plant growth.

Although so far there is no evidence that humic substances affect the electrogenic proton pump operating at the plasmalemma, the simi-

larity of humic substance and auxin effects on plant growth led us to undertake experiments on the effect of sodium humate on the membrane potential in the internodal cells of *Nitellopsis obtusa*. It was found that auxin induces changes in the membrane potential and electric conductivity in giant algal cells (S t o l a r e k 1968). This effect of the auxin on membrane potential and permeability can be considered as being involved in the mechanism of IAA regulatory action on the growth of plant cells (H a g e r et al. 1971).

MATERIAL AND METHODS

The experiments were carried out with internodal cells of *Nitellopsis obtusa* cultured in the laboratory in tap water.

The culture was illuminated 12 hours daily with a combination of incandescent and luminescent light.

The electric potential difference between the vacuole and the external medium was measured with a microelectrode inserted into the vacuole and an electrode placed in the external medium. The microelectrodes were prepared in the routine way from Pyrex glass tubes. The external diameter of the sharp tip was about 1 μm. They were filled with 3 M KCl. The microelectrodes were connected to calomel electrodes. The microelectrodes were inserted into the cells with a Zeiss slide micromanipulator, the movement of the microelectrode tip being observed through the horizontally placed microscope. The recording and reference electrodes were joined to the high-input impedance (10^{13} Ω) amplifier and a chart recorder (Rikadenki K-66, Japan).

The electric resistance was measured with a multimeter V-640 (Unipan, Poland), where the voltage drop across a known resistor and the current flowing in the circuit were recorded. The composition of the ground experimental medium was as follows: 0.1 mM KCl, 1.0 mM NaCl and 0.1 mM $CaCl_2$ referred to as artificial pond water (APW). The concentration of sodium humate used was 12.5, 25, 50 and 100 mg·dm^{-3}.

Na-humate was initially dissolved in 0.5 cm^3 of 0.1 N NaOH, which was then diluted to 1 dm^3 of APW. The concentration of hydrogen ions in the solutions was regulated with MES and HEPES buffer within the range between pH 4.0 and 9.0. The measurements of electric potential differences and resistance between cell interior (vacuole) and external medium were carried out continuously with the cells soaked in the batch containing the given solution. Steady values of membrane potential were obtained 30 min from the moment of exchange of the bathing solution. The solutions in the bath were quickly exchanged with a multichannel tap connecting the trough, containing the cell, with the flasks filled with appropriate solutions through polyethylene tubes.

RESULTS

Administration of sodium humate to the bathing medium brought about hyper- or depolarization of the membrane potential in the internodal cells of *Nitellopsis obtusa*, depending on the concentration of Na-humate. Hyperpolarization was induced by Na-humate of low concentrations (12.5 and 25 mg·dm^{-3}), whereas in higher concentrations (50 and 100 mg·dm^{-3}) this substance caused depolarization (Fig. 1).

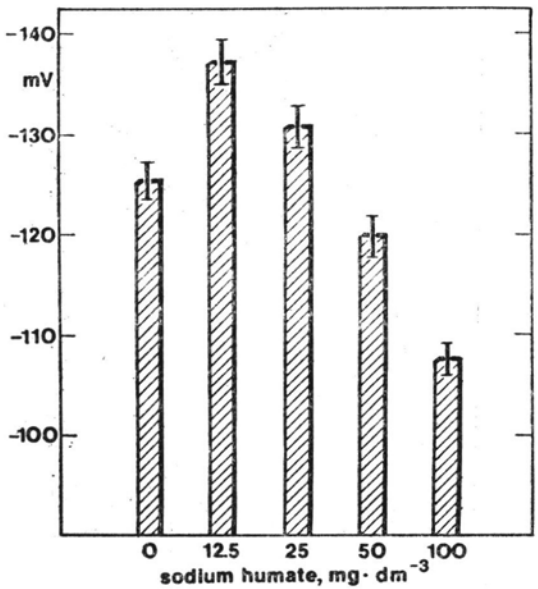

Fig. 1. Effect of Na-humate on the membrane potential difference measured between the vacuole and the external medium in cells of *Nitellopsis obtusa*. The data are means of ten experiments. Vertical bars denote standard error of the mean. The composition of the control solution referred to as APW was as follows: 0.1 mM KCl, 1.0 mM NaCl and 0.1 mM $CaCl_2$

In order to examine the link between H^+ concentration in the medium and Na-humate-induced changes in the membrane potential it was necessary to investigate first the effect of pH of the external medium on the value of the electric potential difference between the vacuole and external media. Fig. 2 shows the dependence of the membrane potential on the acidity of the medium within the range of pH 9.0-4.0. The rise in the acidity of the medium was accompanied by a substantial positive shift of the membrane potential. According to the data shown in Fig. 2 at pH ranging between 5.0 and 4.0 a large depolarization was observed which agrees with the data of other workers (S t o l a r e k 1972). However, the acid pH caused only a slight depolarization of the membrane

potential when Na-humate was present in the bathing medium (Fig. 2, upper curve). The influence of Na-humate on the pH-dependent membrane potential was strong only at pH 4.0 and 5.0, i.e. the sodium humate caused a marked reversal of the depolarization only at low pH values.

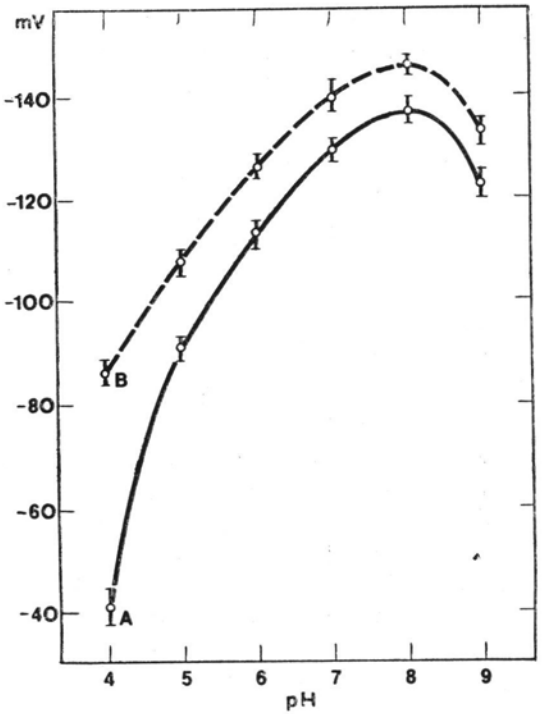

Fig. 2. Effect of pH (A) and Na-humate at different pH values of the bathing medium (B) on membrane potential in *Nitellopsis obtusa*. Each series comprised eight experiments with different cells. Vertical bars denote standard error of the mean. The composition of control medium (APW) was the same as indicated in Fig. 1

The electric resistance measured between the vacuole and the external medium assumed the lowest value at pH 8.0 and gradually increased with the rise of acidity of the external medium (Fig. 3). The addition of Na-humate brought about a rise of the d.c. resistance, which was greater at higher pH values and less pronounced at pH 4.0.

DISCUSSION

The effect of Na-humate on the electric potential difference between the vacuole and the external medium in giant cells of *Nitellopsis obtusa* is similar to the action of auxin (S t o l a r e k 1968, 1972). Nevertheless, Na-humate induced changes in the membrane potential depended speci-

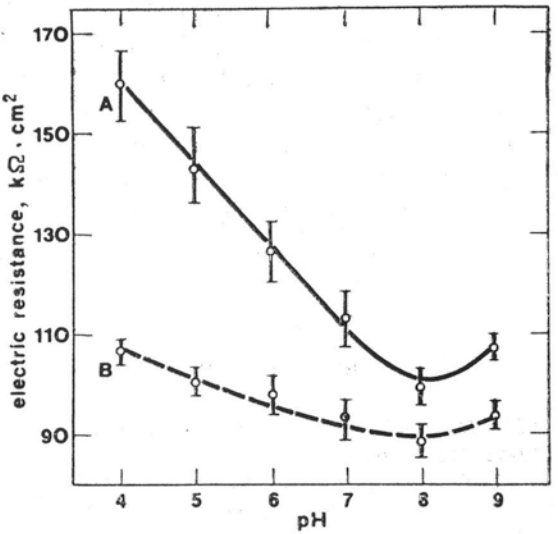

Fig. 3. Electric resistance between the vacuole of *Nitellopsis obtusa* cells and the external medium as a function of pH of the bathing solution APW without Na-humate (A) and in its presence, i.e. APW + 12.5 mg·dm^{-3} of Na-humate (B). Vertical bars denote the standard error of the mean

fically upon the pH of the external medium. Such an effect of humate on the membrane potential is compatible with the findings of Kyć (1970), who found that sodium humate prevented the injurious effects of low pH on the growth of *Scenedesmus*. However, the mechanism of humate action still requires further investigations. Our results supply new evidence in favour of the theory according to which humus exerts a hormone-like action on plant cells. It is noteworthy that the changes in electric resistance between the cell interior and the external medium, induced by Na-humate, resemble those caused by auxin as observed by Stolarek (1968).

Although the nature of Na-humate-induced changes of the membrane potential in giant coenocyte algal cells is unknown, it seems likely that humate alters the permeability of the plasmalemma to major ions determining the electric potential difference across this membrane. Such a view is corroborated by the results of our investigation on the humate effect on electric resistance in cells of *Nitellopsis obtusa* (Fig. 3). The drop in electric resistance caused by humate indicates an increased permeability to some current carrying ions, presumably potassium and hydrogen. An alternative possibility which should be taken into account in order to explain the humate action is the direct effect of this substance on the electrogenic proton pump in the plasmalemma, which may be responsible for the observed membrane potential changes. The operation of active electrogenic proton transport in plasmalemma through the

agency of the proton pump, extruding H^+ out of the cells was found in *Nitella translucens* by Spanswick (1972) and in *Nitellopsis obtusa* by Stolarek (1977) and consecutively by other workers. In view of the fact that the electrogenic proton pump is responsible for the electrical reaction of plant cells to the action of light, auxin and other phytohormones (Zientara 1982), fungal toxins (e.g. fusicoccin) etc. it is probable that the primary effect of Na-humate on plants consists in regulation of the ionic permeability of the plasmalemma, especially the proton pump.

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Działanie humianu sodu na potencjał membranowy międzywęzłowych komórek Nitellopsis obtusa

Streszczenie

Zbadano działanie czterech dawek humianu sodu: 12,5, 25, 50 i 100 $mg \cdot dm^{-3}$ na potencjał membranowy komórek *Nitellopsis obtusa* stwierdzając, że pierwsza z nich powodowała najwyższą hyperpolaryzację potencjału elektrycznego. Następnie

przebadano wpływ 12,5 mg·dm^{-3} humianu sodu na potencjał membranowy i opór elektryczny komórek glonu w zależności od pH środowiska zewnętrznego. Stwierdzono, że humian sodu powodował nie tylko hyperpolaryzację potencjału membranowego komórek *Nitellopsis obtusa* ale też wykazywał swoistego rodzaju działanie ochronne widoczne najwyraźniej w pożywce o wysokiej kwasowości (pH 4-5). Humian sodu wprowadzony do środowiska powodował ponadto spadek oporu elektrycznego komórek glonu zmniejszając jednocześnie jego wrażliwość (w całym zakresie pH) na zmiany kwasowości pożywki. Wyniki niniejszej pracy sugerują, że pierwotne działanie humianu sodu polega na regulacji przepływu jonów na poziomie plazmalemmy, w szczególności zaś substancja ta może stymulować pompę protonową.