ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. 52, nr 3-4: 279-284
1983

Effect of auxin, gibberellin and kinetin on membrane potential and proton pump in wheat coleoptile cells

MARIA ZIENTARA

Department of Plant Physiology, Silesian University,
Jagiellońska 28, 40-032 Katowice, Poland

(Received: February 21, 1983. Revision accepted: May 16, 1983.)

Abstract

The changes in the electric potential differences between the vacuole and the external medium, induced by IAA, GA_3 and kinetin in wheat coleoptiles were investigated in relation to the acidification of the external medium brought about by these growth regulators. IAA caused a greater hyperpolarization of the membrane potential and larger enhancement of the proton pump as compared with GA_3, whereas kinetin exerted no effect. Ouabain partly reversed IAA-induced hyperpolarization, this suggesting that ATPase might be involved in the action of IAA on ion transport processes.

Key words: auxin, gibberellin, cytokinin, proton pump, membrane potential.

INTRODUCTION

β-indole acetic acid (IAA) stimulates proton extrusion and potassium uptake leading to a shift in the membrane potential in plant cells (Hager et al. 1971). A relationship exists between IAA action, proton extrusion and elongation growth (Cleland and Rayle 1978, Cocucci and Dalla Rosa 1980, Marrè 1977a, b). It has been established that auxin activates cell elongation by stimulating the proton pump located in the plasmalemma. The question arises whether this mode of action of IAA is valid in respect to other major phytohormones, namely, gibberellin and kinetin. So far, however, there are no data in the literature concerning the action of gibberellin and kinetin on membrane potential and proton transport in higher plant cells. Therefore, it seemed worthwhile to undertake comparative investigations of the effect of IAA, gibberellin and kinetin on both membrane potential and proton extrusion in cells of the same plant tissue such as the coleoptile.

MATERIAL AND METHODS

The experiments were carried out with four-day-old coleoptiles of *Triticum vulgare* var. Dańkowska. The seedlings were grown in Petri dishes in darkness. Measurements of the membrane potential between the vacuole and external solution in the coleoptile cells were performed with the use of the microelectrode method fully described in a previous paper (S t o l a r e k 1972). The membrane potential was first measured in cells surrounded by the solution designated as APWK the following composition: 0.1 mM NaCl, 1.0 mM KCl, then the coleoptiles were soaked in APWK containing IAA, gibberellin or kinetin. The concentration of the phytohormones in the external solution was 10^{-5} M. Twenty four hours later the membrane potential was recorded again. The acidification of the medium was also followed during 24 hours both in the control batch of coleoptiles soaked in APWK and in APWK containing IAA, kinetin or gibberellin, respectively. Each sample consisted of 25 coleoptile segments soaked in 6 dm^{-3} of incubation medium. The length of the segments was 10 mm.

pH was measured with a Radelkis OP-205 pH-meter. During the measurements the external solution was constantly stirred with a magnetic stirrer. The apparatus consisted of glass microelectrodes, calomel electrodes joined to a high input impedance d.c. amplifier, a high-speed multichannel recorder (Rikadenki KB-66, Japan) and a voltmeter V-640 (Unipan). The microelectrodes were filled with 3 M KCl solution. The microelectrode was inserted into the cell with the use of a Zeiss micromanipulator. The cell interior and the tip of the microelectrode were observed in a light microscope during the measurements.

RESULTS

The results of the experiments on acidification of the medium induced by auxin, gibberellin or kinetin are listed in Table 1. Each result is a mean of six measurements on separate coleoptiles. It was found that the greatest stimulatory effect on proton ejection was brought about by IAA, a smaller stimulation was produced by gibberellin and only a slight enhancement of proton extrusion was noticed in the case of kinetin.

The action of IAA, gibberellin and kinetin on the membrane potential is shown in Table 2. It follows from the results that IAA and gibberellin induce hyperpolarization amounting to 18 and 8 per cent, respectively as compared with the control. Practically no effect of kinetin on the membrane potential was noticed.

Table 1

Effect of IAA, gibberellin and kinetin (10^{-5}) on the acidification of the medium by wheat coleptiles

Series	pH of the medium		ΔpH	ΔpH $\cdot g^{-1}$ fr. wt.
	initial	after 24 h		
Control	7.00±0.011	6.62±0.020		
IAA	6.99±0.008	6.33±0.028	0.29	0.95
Control	7.00±0.013	6.75±0.016		
Gibberellin	7.02±0.009	6.63±0.013	0.12	0.39
Control	7.00±0.013	6.72±0.010		
Kinetin	7.02±0.011	6.77±0.027	0.05	0.16

The data are mean values from 15 experiments. The figures marked with ± denote the standard error of the mean

Table 2

Effect of IAA, gibberellin and kinetin on membrane potential of wheat coleoptile cells

Series	Membrane potential, mV	Per cent of the control
Control	52.8±1.117	
IAA	61.2±1.693	117.8
Gibberellin	57.1±1.458	108.1
Kinetin	53.6±1.515	101.5

Each result is a mean value from 15 experiments. The figures marked with ± denote SEM.

Table 3

Effect of IAA and ouabain on membrane potential in wheat coleoptile cells

Series	Membrane potential, mV	Per cent of the control
Control	52.6±1.210	
IAA	62.4±1.693	118.0
Ouabain	47.7±1.439	90.6
IAA+ouabain	55.2±1.520	105.0

15 experiments have been performed in each series. The figures marked with ± refer to SEM.

Ouabain known as an inhibitor of ion-stimulated ATPase caused depolarization of the membrane potential; this effect being partly reversible by IAA (Table 3).

DISCUSSION

Stolarek (1968, 1972) has shown that in cells of *Characeae* IAA induces changes in electric potential differences between the vacuole and the external solution. The change in membrane potential was simultaneously accompanied by a large drop in transmembrane electric resistance. The author postulates that IAA stimulated active electrogenic transport of cations across the plasmalemma, which is responsible for changes in the electric parameters of the membranes. It was also suggested that IAA enhances ATPase activity because ouabain, an inhibitor of transport ATPase, partly reversed the effect of IAA.

The results obtained in the present work gave further evidence in favour of the membrane mechanism of IAA action in higher plants (Stolarek 1968, Hager et al. 1971, and others). This view is corroborated by our finding that IAA at a concentration of 10^{-5} M induces both hyperpolarization and proton extrusion in the cells of wheat coleoptiles (Tables 1 and 2). The IAA-induced hyperpolarization and proton extrusion in the cells of wheat coleoptiles is similar to that in barley coleoptiles (Stolarek and Zientara 1983). The hyperpolarization induced by IAA is probably caused by enhancement of the proton pump ejecting H^+ ions from the cells. Nelles (1978) reported that a fungal toxin fusicoccin induced a high hyperpolarization in maize coleoptiles. He postulated that this effect is due to the stimulatory action of IAA on the proton pump in the plasmalemma, which extrudes hydrogen ions. The results of our experiments with wheat coleoptiles corroborate such a view.

Two other hormones, gibberellin and kinetin, differ in their action on the membrane potential and proton extrusion.

Our findings lead to the conclusion that IAA and gibberellin act on the membrane permeability in a similar way, whereas kinetin does not affect either the membrane potential or the proton pump. This fact might constitute a significant point as far as the mechanism of action of these major phytohormones is concerned.

Partial reversal of IAA induced hyperpolarization by ouabain which is known to be an inhibitor of membrane-bound ATPase in plant cells points to the involvement of ATP as energy source for the stimulatory effect of IAA on the proton pump.

Acknowledgment

The financial aid of the Committee of Biochemistry and Biophysics of the Polish Academy of Sciences is gratefuly acknowledged. I would like to express my gratitude to Doc. Dr hab. Jan Stolarek for a helpful discussion.

REFERENCES

Cleland R. E., Rayle D. L., 1978. Auxin, H^+-excretion and cell elongation. Bot. Mag. Tokyo Special Issues 1: 125-139.

Cocucci M., Dalla Rosa S., 1980. Effects of canavanine on IAA and fusicoccin-stimulated cell enlargement, proton extrusion and potassium uptake in maize coleoptiles. Physiol. Plant. 48: 239-242.

Hager A., Menzel H., Krauss A., 1971. Versuche and Hypothese zur Primärwirkung des Auxins beim Streckungswachstum. Planta 100: 47-75.

Marrè E., 1977a. Effects of fusicoccin and hormones on plant cell membrane activities: observations and hypotheses. In: Regulation of cell membrane activities in plants. E. Marrè, O. Cifferi (eds.), North Holland Publ. Co., Amsterdam. pp. 185-202.

Marrè E., 1977b. Physiologic implications of the hormonal control of ion transport in plants. In: Plant growth regulation. P. E. Pilet (eds.), Springer-Verlag, Berlin-Heidelberg-New York, pp. 54-66.

Nelles A., 1978. Evidence for a fusicoccin-stimulated potassium ion pump in cell membranes of dwarf maize coleoptiles. Plant Sci. Letters 12: 349-354.

Stolarek J., 1968. The effect of IAA on bioelectric potentials and resistance in giant plant cells. Studia Biophys. 6: 187-192.

Stolarek J., 1972. Niektóre aspekty transportu i zjawisk elektrycznych w komórkach roślinnych. D. Sc. Thesis. M. Curie-Skłodowska University, Lublin.

Stolarek J., Zientara M., 1983. The effect of auxin and gibberellin on membrane potential in coleoptile cells of a tall and dwarf strain of barley. Acta Biologica 11: 43-52.

Działanie auksyny, gibereliny i kinetyny na potencjał membranowy i pompę protonową w komórkach koleoptyli pszenicy

Streszczenie

Zbadano wpływ trzech hormonów: auksyny, gibereliny i kinetyny w stężeniu 10^{-5} M na zakwaszanie środowiska zewnętrznego i potencjał membranowy komórek koleoptyli *Triticum vulgare*, odmiany Dańkowska. Stwierdzono, że auksyna powodowała ponad dwukrotnie silniejsze wyrzucanie jonów H^+ do środowiska zewnętrznego w porównaniu z gibereliną, natomiast kinetyna pozostawała bez wpływu na ten proces. Auksyna indukowała również najwyższą hyperpolaryzację potencjału membranowego — 17,8%, giberelina — 8,1% a kinetyna zaledwie — 1,5%. Tak więc hyperpolaryzacja potencjału membranowego wywołana działaniem auksyny, jest spowodowana prawdopodobnie, nasileniem wyrzucania jonów wodorowych przez pompę protonową na zewnątrz komórki. Wyniki doświadczeń pro-

wadzą do konkluzji, że działanie auksyny i gibereliny na przepuszczalność błon jest bardzo podobne, natomiast kinetyna nie wpływa na aktywność pompy protonowej i potencjał membranowy. Częściowe odwrócenie indukowanej auksyną hyperpolaryzacji potencjału membranowego, spowodowane przez ouabainę, która jest znanym inhibitorem ATPazy związanej z błonami komórek roślinnych, świadczy o włączeniu ATP jako źródła energii dla stymulującego działania auksyny na pompę protonową.