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Ionic relations and electrophysiology of single cells of *Characeae*

Part IV. The effect of IAA on bioelectric potentials in single cells of *Characeae*

J. STOLAREK

INTRODUCTION

A link between the effect of auxin and electric polarity of plant tissues was established a long time ago in investigations on the mechanism of photo- and geotropic reactions. Chołodny (1947) and Went (1928) found that the phototropic bend of the oat coleoptiles towards light could be explained by a horizontal transport of auxin from the illuminated to the dark side of the organ. Greater concentration of auxin on the dark side leads to a faster growth of the cells which results in the production of a bend of the coleoptile tip towards the source of light.

Van Overbeek found that the total auxin content in the illuminated coleoptile is the same as in the coleoptile kept in darkness. Thus the horizontal gradient of the hormone concentration must be connected with its transverse transport. The mechanism of polar auxin transport has not been explained as yet. A first hypothesis was proposed by Brauner (1927) suggesting that auxin movement in plants is controlled by bioelectric potentials. This hypothesis was corroborated by Lund (1947) and Schrank (1951) who established that there is a longitudinal gradient of bioelectric potential and the tip is electrically negative as compared with the base. Siniuchin and Stolarek (1961) extended these investigations on the coleoptiles of *Zea mays*. Auxin produced in the coleoptile tips moves in basipetal direction, i.e. towards the positively charged zone. Moving as an anion auxin could be transported cataphoretically. In a laterally illuminated coleoptile the dark side becomes electrically positive as compared with the illuminated one (Bose 1907).

The formation of the geotropic curvature is accompanied by an electric polarisation — the lower side of the coleoptile becomes electrically negative as compared with the upper surface. In both cases auxin moves towards the positively charged region. Electric polarisation precedes the production of photo and geotropic curvatures and it gradually disappears at the same rate as the curvature is formed. Webster and Schrank (1953) concluded that appearance of electric polarisation is the cause of auxin transport in photo and geotropic reactions. The existence of transverse auxin transport during photo- and geotropic reactions was established by Hertel with C^{14} labelled auxin (IAA).

Schrank (1953) suggested that the first event in geo- and phototropic reactions is the change in electric potentials, which brings about the movements and appropriate distribution of IAA.

Scott (1967) showed that in roots of *Vicia* electric potentials oscillated with a frequency of 12 hr^{-1}. He suggested that a feedback control pathway in roots is over-corrected with the following sequence of events: bioelectric current through the tissue influences the distribution of auxin to sites where it affects membrane permeability, it may could alter membrane potentials and the bulk current flow, thus completing the feedback loop.

Newman (1963, 1965) demonstrated that the application of IAA on top of a decapitated *Avena* coleoptile brought about the appearance of an electric wave travelling down the organ at the speed of auxin transport. Grahm and Hertz (1964) showed that the asymmetric application of IAA on top of the decapitated coleoptile induced lateral potentials close to those accompanying the geotropic response. The ability to produce a geotropic bend in decapitated coleoptiles disappears first near the cut and then spreads down the coleoptiles at a speed of 15 mm/hr. corresponding to the rate of IAA movement. All these works supply evidence that there is a close interrelation between IAA availability and electric activity in plant tissues.

The purpose of the present paper is the investigation of the effect of IAA on bioelectric potentials in the internodial cells of *Characeae*. The experiments of Stolarek (1968) showed that IAA in a physiological concentration increased potassium influx in *Nitella translucens*. Thus it seemed reasonable to undertake investigations on the effect of IAA on electric potential differences between the vacuole and the external medium.

MATERIAL AND METHODS

Experiments were performed on cells of *Nitella syncarpa*, *Nitella translucens*, *Chara fragilis* and *Lychnothamus barbatus*. The cells were cultured in the laboratory.

Bioelectric potential differences were measured with the use of "Pyrex" microelectrodes filled with 3 M KCl, a d.c. amplifier with a high input impedance and a recorder or galvanometer. The potential difference was measured between the vacuole and the external medium. The experiments were carried out on cells bathed with an external solution called "artificial pond water" or "APW" of the following composition: 0.1 mM KCl, 0.1 mM $CaCl_2$, 1.0 mM NaCl with IAA (10^{-4}M, 10^{-5}M) or without IAA. pH of the solutions containing IAA was adjusted with phosphate buffer, which was found to have no effect on the potential difference measured.

RESULTS

a) Effect of IAA on the bioelectric potentials of internodial cells of Characeae. The results of the measurements performed on 15 control cells of *Nitella translucens* showed that the potential difference between the vacuole and external medium as a function of time is almost a straight line i.e. electric potential in these conditions is almost constant. In some cases a depression in potential difference was found

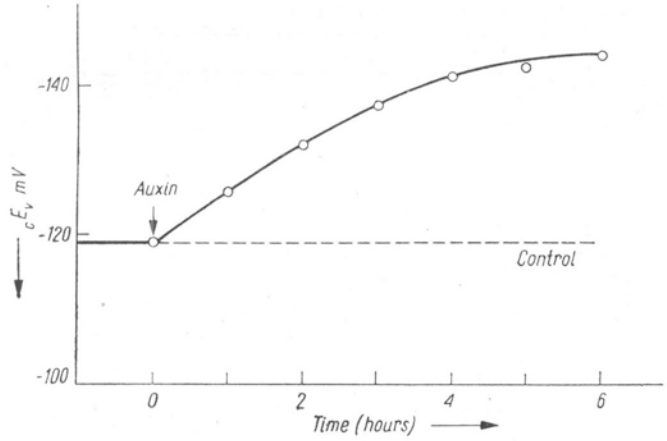

Fig. 1. Effect of indolylacetic acid (IAA) on electric potential difference between vacuole and external medium in 14 cells of *Nitella translucens*

between 9 p.m. and 3 a.m. o'clock. The concentrations of IAA used were 2.10^{-4} and 2.10^{-5}M. The cells were kept first in APW, then the medium was changed for APW+IAA. It was found that IAA at a concentration of 2.10^{-5}M brought about an increase in potential difference between the vacuole and the medium in all the investigated species, whereas a concentration of 2.10^{-4}M led to a drop in the potential difference (Tab. 1).

The increase in the potential difference between the vacuole and the medium as a result of the effect of IAA on cells of *Nitella translucens* amounted to −7 mV after 1 hour and −24 mV after an 6 hours' exposure. A tenfold higher concentration of IAA (2.10^{-4}M) induced a decrease in the potential difference from +8 mV after 1 hour to +23 mV after 6 hours' exposure. The corresponding figures for other species are: *Chara fragilis* −9 mV (2.10^{-5}M IAA) and +18 mV (2.10^{-4}M) *Lychnothamus barbatus* −20 mV and +19 mV. The value of the change in potential difference (△E) was obtained from the comparison of the potential difference at a given time with that at zero time, recorded in cells soaked in APW (Table 1, Fig. 1).

The effect of IAA on the bioelectric potentials of plant cells could be connected with the changes in the permeability of the cytoplasm for ions determining the

Table 1

Effect of IAA on bioelectrical potential difference between the vacuole and external medium in cells of *Lychnothamus barbatus*, $_{v}E_{0}$ (mV)

Expt.	Number of cells	Potential difference between vacuole and external medium when cell is bathed in the following solution:												
		Artificial pond water (A.P.W.) Control	A.P.W.+IAA ($2\cdot10^{-4}$ M) Cells soaked:				A.P.W.+IAA ($2\cdot10^{-5}$ M) Cells soaked:							
			1 hr		6 hrs		1 hr		2 hrs		4 hrs		6 hrs	
		E	E	△E	E	△E	E	△E	E	△E	E	△E	E	△E
1		—117					—134	—17	—145	—28	—153	—36	—153	—36
2		—121					—128	—7	—134	—13	—145	—24	—147	—26
3		—107					—120	—13	—116	—9	—120	—13	—124	—17
4		—127					—137	—10	—137	—10	—142	—15	—145	—18
5		—127					—133	—6	—136	—9	—137	—20	—140	—13
6		—116					—114	+2	—124	—8	—134	—18	—140	—24
7		—118					—122	—4	—122	—4	—130	—16	—135	—17
8		—120					—114	+6	—136	—16	—140	—20	—145	—25
9		—120					—124	—4	—124	—4	—145	—25	—146	—26
10		—116					—133	—17	—137	—21	—143	—27	—146	—30
11		—118					—134	—16	—116	+2	—150	—32	—150	—32
12		—121					—128	—7	—145	—24	—146	—25	—150	—29
13		—117					—122	—5	—134	—17	—145	—28	—150	—33
14		—123					—123	0	—140	—17	—145	—22	—147	—14
Mean ±s.e.m.		—119 ±2.0					—126 ±2.15	—7,0 ±1.43	—132 ±2.7	±12.7 ±2.24	—141 ±2.26	—23.6 ±1.74	—144 ±2.01	—24.3 ±2.28
Mean ±s.e.m.	10	—118.0 ±2.0	—110 ±2.1	+8.0 ±1.5	+9.0 ±2.0	+23 ±1.2								
		Measurements carried out on control batch of cells												
Mean ±s.e.m.	15	—124.0 ±1.0					—124 ±1.0		—124 ±1.0		—127 ±1.0		—124 ±1.2	

Table 2

Effect of IAA and Na^+, K^+, Ca^{2+}, Cl^+ ions on bioelectric potentials in cells of four Characeaen species

The sequence of the solutions	Concentration of ions (mM)						Changes in ion concentration				The changes in electric potential difference between vacuole and external medium (mV)							
											In the media without IAA				In the media containing IAA ($2 \cdot 10^{-5}$ M)			
	Na	K	Ca	Cl	Choline	Benzene sulphonate	Na	K	Ca	Cl	*Chara fr.*	*Lychnothamus*	*Nitella transl.*	*Nitella syncarpa*	*Chara fragilis*	*Lychnoth. barb.*	*Nitella transl.*	*Nitella syncarpa*
B1	0.1	0.1	0.1	0.4			+1.0			+3.25	+6.0	+8.0	+7.3		+5.0	+9.0	+7.0	
APW	1.0	0.1	0.1	1.3			x			x	±1.5 (10)	+0.5 (10)	±1.2 (20)		±0.3 (10)	±0.5	(10)	
APW	1.0	0.1	0.1	1.3			—10	+10			+5.0	+3.0	+4.0		+10.0	+7.0	+7.0	
B3	0.1	1.0	0.1	1.3			x	x			±1.5 (10)	±1.5 (10)	±1.5 (20)		±0.4 (12)	±0.6 (9)	(9)	
B3	0.1	1.0	0.1	1.3				—10		—3.25	—7.0	—8.0	—9.3		—20.0	—18.0	—15.0	
B1	0.1	0.1	0.1	0.4				x		x	±0.6 (10)	±0.6 (10)	±1.9 (20)		±1.2 (15)	±1.1 (9)	(10)	
B1	0.1	0.1	0.1	0.4					+10	+5.5	+13.0	+5.0	+15.0		+18.0	+10.0	+15.0	
B2	0.1	0.1	1.0	2.2					x	x	(10)	(10)	(19)		(10)	(9)	(7)	
B2	0.1	0.1	1.0	2.2			+10			+1.4	0	0	+0.7		+2.0	+2.0	+1.0	
B6	1.0	0.1	1.0	3.1			x			x	(5)	(6)	(9)		(5)	(5)	(5)	
B1	0.1	0.1	0.1	0.4						+8.5	—7.0	+4.0	—6.0	—5.0	—8.0	—5.0	—7.0	—5.0
B4	0.1	0.1	0.1	3.4	3.0					x	(10)	(5)	(10)	(20)	(10)	(5)	(10)	(10)
APW	1.0	0.1	0.1	1.3						—6.5	+7.0	+3.0	+5.5	+4.0	+9.0	+6.0	+4.0	+6.0
B5	1.0	0.1	0.1	0.2		1.1				x	(10)	(5)	(20)	(20)	(10)	(5)	(8)	(5)

The figures in brackets denote the number of examined cells. Some of the results are given with s.e.m. (±).

bioelectric potential differences, i.e. for potassium, sodium, calcium and possibly chloride.

Another series of measurements was done in order to investigate the effect of ions present in the medium on bioelectric potential difference in the cases when IAA was present in the medium or absent. The presence of IAA in the medium changes the reaction of bioelectric potential of the cell to tenfold increase in potassium concentration (underlined data in Tab. 2). In the presence of IAA (2.10^{-5}M) the changes in potential difference corresponding to the sequence of the solutions APW — B3 (tenfold increase of potassium concentration in the medium) were

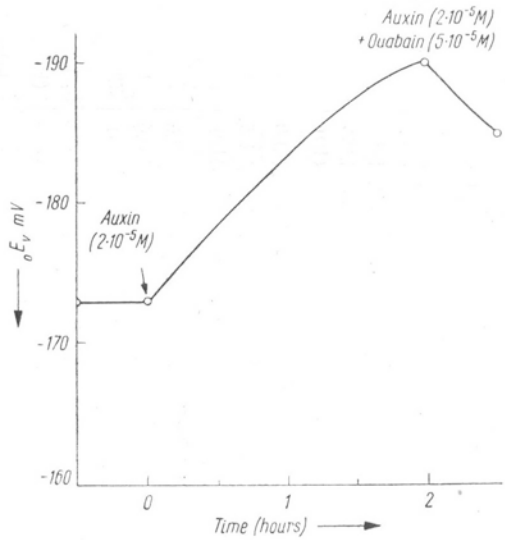

Fig. 2. Effect of auxin and ouabain on electric potential difference between vacuole and external medium in cells of *Chara fragilis*

as follows: for *Chara fragilis* +10 mV, that is twice as high as the +5 mV change corresponding to the same sequence of solutions but without IAA. The effect of the changing concentrations of other ions in the media containing IAA is essentially the same as without IAA.

b) Effect of ouabain on bioelectric potentials of cells of *Nitella translucens*, *Chara fragilis* and *Lychnothamus barbatus*. MacRobbie (1962) found that ouabain inhibits potassium influx (by 40%) in *Nitella translucens*. Stolarek (1968) demonstrated that ouabain inhibits the stimulatory effect of IAA on potassium uptake in *Nitella translucens* and also that ouabain does not affect the potential difference in these cells.

The joint effect of IAA and ouabain was studied in the following way: first the potential difference between the vacuole and the medium in APW (control) was recorded, then the medium was changed for APW+IAA (2.10^{-5}M) and after the lapse of 2 hours the potential difference was recorded again. The increase in

Table 3

Effect of IAA and ouabain on bioelectrical potential in cells of *Nitella translucens*, *Chara fragilis* and *Lychnothamus barbatus* (mV).

Species	Cell	Potential difference between the vacuole and external medium recorded when cell was bathed by one of the following solution:				
		"Artificial pond water" (A.P.W.) Control	A.P.W. + IAA ($2 \cdot 10^{-5}$ M), 2 hours of soaking		A.P.W. + IAA ($2 \cdot 10^{-5}$ M) + ouabain ($5 \cdot 10^{-5}$ M), 30 min. of soaking.	
			E	△E (4—3)	E	△E (6—4)
1	2	3	4	5	6	7
Nitella translucens	1	—117	—132	—15	—122	9
	2	—120	—135	—15	—130	5
	3	—118	—128	—10	—116	12
	4	—121	—136	—15	—128	8
	5	—122	—138	—16	—127	11
	6	—127	—145	—18	—135	12
	7	—123	—130	—7	—119	11
	8	—118	—128	—10	—118	10
	9	—118	—132	—14	—121	11
	10	—127	—136	—9	—126	10
	Mean ±	—121	—134	—13	—124	10
	s.e.m.	1.16	1.7	1.14	1.87	0.60
Lychnothamus barbatus	1	—175	—180	—5	—175	5
	2	—165	—172	—7	—166	6
	3	—162	—170	—8	—163	7
	4	—173	—181	—8	—175	6
	5	—174	—185	—11	—176	9
	6	—169	—175	—6	—170	5
	7	—164	—180	—14	—168	12
	8	—163	—171	—9	—162	9
	9	—161	—177	—16	—162	15
	10	—170	—186	—16	—171	15
	Meam ±	—168	—181	—10	—169	9
	s.e.m.	1.7	2.1	1.29	1.76	1.22
Chara fragilis	1	—175	—188	—13	—176	12
	2	—178	—195	—17	—190	15
	3	—180	—190	—10	—184	6
	4	—174	—188	—14	—178	10
	5	—179	—190	—11	—199	9
	Mean ±	—173	—190	—13	—185	10
	s.e.m.	2.6	1.22	1.22	4.20	1.52

potential difference for *Lychnothamus barbatus*, *Chara fragilis* and *Nitella translucens* was respectively −10, −13 and −13 mV. Then the new solution consisting of APW+IAA (2.10^{-5}M)+ouabain was given. The potential difference decreased (became more positive) (Tabl. 3). The time course of the changes in potential difference in cells pretreated with external solutions containing both IAA and ouabain is shown in Fig. 2.

DISCUSSION

From the data presented in this paper it appears that IAA brings about changes in the electric potential difference between the interior of the cells and external solution. It was found that IAA stimulated potassium uptake in *Nitella translucens*, and the effect of ouabain on potassium uptake suggests that IAA stimulates passive as well as active influx. An increase of passive influx means that the permeability coefficient for potassium P_k increases and the coefficient $\alpha = P_{Na}/P_K$ in the Goldman equation increases, since P_{Na} does not change when IAA is added to the medium (Stolarek 1968).

Knowing the increase in potassium permeability induced by IAA it is possible to calculate the increase in the electric potential difference between the cytoplasm and external medium induced by IAA. In order to give a quantitative discussion of the results it is necessary to recall the data concerning the changes in potassium permeability induced by IAA (Stolarek 1968). (Potassium influx is expressed in pM. cm^{-2}. sec^{-1}).

1. K^+influx in control cells (soaked in APW) $M^i_K = 0.732$

2. K^+influx in the solution: APW+ouabain, $M^i_{K,p} = 0.419$. This is the passive component of the influx.

3. K^+influx measured in the mediun: APW+IAA (2.10^{-5}M), $M^i_{K,a} = 1.224$

4. K^+influx measured in the solution APW+IAA (2.10^{-5}M)+ouabain, $M^i_{K,a,s} = 0.732$. This is the passive component of the potassium influx induced by IAA. MacRobbie (1962) used a simplified equation for K^+influx in cells of *Nitella translucens*:

$$M^i_K = \frac{FE}{RT} P_K \cdot K_0 \qquad (1)$$

where E — potential difference between the cytoplasm and the external solution. P_K is the permeability coefficient of the cytoplasm for potassium ions, K_0 — potassium concentration in external solution, R and T have their usual meaning. From expression (1) we obtain

$$P_K = \frac{M^i_K}{K_0} \frac{RT}{FE} \qquad (3)$$

The ratio of the permeability coefficients for potassium in control conditions P_K

to $P_{K,a,s}$ (permeability coefficient for cell soaked in the solution containing IAA and ouabain) is equal to the ratio of influxes in both cases:

$$\frac{P_{K,a,s}}{P_K} = \frac{M^i_{K,pas}}{M^i_{K,a,s}} = \frac{0.419}{0.724} = 0.543$$

It is evident that the permeability coefficient of the cytoplasm for potassium ions increases two times in the medium with IAA compared with the control. It has been shown elsewhere that IAA does not affect sodium influx in *Nitella translucens*. The value of in cells treated with IAA is equal to

$$\alpha_a = P_{Na}/P_K \cdot 0.543 = 0.543\ \alpha$$

where $\alpha = P_{Na}/P_K$ in the control solution (APW) $\alpha_a = \frac{P_{Na}}{P_{K,a,s}}$ in the solution containing auxin and ouabain. It follows from the Goldman equation that

$$E - E_a = 581 \text{ og } \frac{K_{01} + \alpha Na_{01}}{K_{02} + \alpha Na_{02}}$$

where E — potential difference between the cytoplasm and external medium measured in cells bathed with the control solution (APW), E_a — potential difference in cells treated with IAA. Taking $\alpha = 0.27$ for *Nitella translucens* (Stolarek 1968) we obtain: $\triangle E = E - E_a = 10$ mV. On the other hand IAA increased potential difference by −23 mV in *Nitella translucens* after 6 hours' exposure i.e. the observed increase in potential difference brought about by IAA exceeded twice the calculated value. This discrepancy might be explained if we assume that IAA caused the increase in both passive and active potassium influx. However, this increasing potassium active influx may be electrogenic. The electrogenicity of active potassium influx in the medium with IAA is corroborated by the results of the experiments on the simultaneous effect of IAA and ouabain on bioelectric potential difference between the vacuole and the external medium (Fig. 2). Ouabain in a concentration of 10^{-5}M added to the control external solution containing IAA caused a drop in potential (Tab. 3). For example in *Nitella translucens* this decrease was 10 mV. IAA increased the potential difference by −20 mV, whereas the predicted value for this change is −10 mV. Ouabain reduced active potassium influx and at the same time decreased the potential difference by −10mV, what would mean that active potassium influx induced by IAA is electrogenic. It is worth mentioning that ouabain added to the control medium i.e. to the solution which did not contain auxin did not affect the electric potential, though it inhibited active potassium influx.

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SUMMARY

The effect of IAA on bioelectric potential differences between the vacuole and the medium in cells of four species of *Characeae*: *Nitella translucens*, *Nitella syncarpa*, *Chara fragilis* and *Lychnothamus barbatus* was investigated. It was established that IAA at a concentration of 2.10^{-5} M caused an increase in electric potential differences by —20 mV, whereas ten times higher concentration of IAA caused a drop in potential differences. Ouabain, an active potassium transport inhibitor when added to the medium containing IAA caused a drop in potential difference by 10 mV, while it had no effect on potential when present in the medium not containing IAA. It was concluded that active transport of potassium ions is electrogenic. Relevant calculations on the basis of the Goldman equation are also given.

Department of Plant Physiology
Biophysical Laboratory
Lublin, ul. Akademicka 12, Poland

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Stosunki jonowe i elektrofizjologia pojedynczych komórek Characeae

Cz. IV. Wpływ IAA na potencjały elektryczne internodialnych komórek ramienic

Streszczenie

Badano wpływ IAA na różnicę potencjału elektrycznego między wodniczką a środowiskiem w czterech gatunkach ramienic: *Nitrilla translucens*, *Nitella syncarpa*, *Chara fragilis* i *Lychnothamus barbatus*. Stwierdzono, że pod wpływem IAA w stężeniu 2.10^{-5} M potencjał elektryczny wzrasta o około —20 mV, podczas gdy 10-krotnie wyższe stężenie IAA powoduje spadek różnicy potencjałów. Ouabaina- inhibitor aktywnego transportu jonów potasu, dodany do pożywki zawierającej IAA powoduje spadek różnicy potencjałów o ok. 10 mV., podczas gdy dodany do pożywki kontrolnej (bez IAA) nie wywiera żadnego wpływu na potencjał elektryczny. Na tej podstawie przyjęto, że aktywny transport jonów potasu jest elektrogeniczny. W pracy przeprowadzono także odpowiednie obliczenia oparte o równania Goldmana.