Ionic relations and electrophysiology of single cells of *Characeae*

Part I. Investigations on electric potentials and resistance in cells of *Nitellopsis obtusa*

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INTRODUCTION

The internodal cells of *Nitellopsis obtusa* were used as a material in the study of ion transport by J. Dainty and E. MacRobbie (1958). This work is widely known and often quoted in the literature. The authors succeeded in achieving a detailed analysis of passive and active transport of potassium, sodium and chloride ions. Their conclusions concerning electrochemical potential differences for these ions across the plasmalemma and tonoplast differ from the data obtained on the cells of other *Characeae* species (Spanswick, Stolarek and Williams, 1967). The electric resistance calculated from ionic fluxes is $250 \text{ k}\Omega \cdot \text{cm}^2$, a value ten times higher than in other species of *Characeae*. It should be noted that so far electric potentials and resistance of cell membranes of *Nitellopsis obtusa* have not been measured, though the well known calculated figures of electric resistance should be compared with the measured values.

The electric resistance across plasmalemma calculated from ionic fluxes is ten times higher than that measured, with microelectrode technique. However, these measured values were obtained on other *Characeae* species (*Chara australis*, *Nitella translucens*) but not on *Nitellopsis* itself. The problem dealt with in this paper is the widely known discrepancy between calculated and measured values of d.c. resistance in plant cells. Thus it seemed reasonable to carry out investigations on electric resistance as well as on electric potential differences in the cells of *Nitellopsis obtusa*.

The purpose of the present study is therefore comparison of the theoretical value of the resistance with the measured one and investigations on the bioelectric potentials and the effect of Na^+ , K^+ and Ca^{2+} ions on it in the cells of *Nitellopsis obtusa*.

MATERIAL AND METHODS

The cells of *Nitellopsis obtusa* were collected in autumn and kept in the laboratory in a solution of the following composition: 0.1 mM KCl, 0.1 mM CaCl_2 and 1.0 mM NaCl denoted as "artificial pond water" and further referred to as "APW". One to three days before the experiments

Fig. 1. The block diagram of the apparatus used for the measurement of electric potential difference between the vacuole and external medium

E_{v1} — microelectrode inserted into the vacuole; E_{v2} — reference calomel electrode placed in the medium; G — galvanometer GES-2A

internodal cells were cut from the stems. Isolated cells did not show any sign of damage and the protoplasmic streaming in them was as intensive as in intact cells. After isolation the cells remained alive for a long time (several weeks). Internodal cells of *Nitellopsis obtusa* are very suitable material for electrophysiological studies not only owing to their regular cylindrical shape but also because the protoplasmic streaming in them — an excellent characteristic of their vitality — is easily seen under the microscope.

Electric potential difference was measured with the use of glass microelectrodes joined to a d.c. amplifier with a very high input impedance ($10^{14} \Omega$) and a GES2A galvanometer. The sensitivity of the apparatus was 2 mV/one division of the scale. The zero drift of the amplifier did not exceed 1 mV/hour. Glas microelectrodes were filled with 3M KCl and joined to calomel electrodes by pieces of rubber tubing. The potential difference measured was that between the vacuole and external medium and was recorded with the microelectrode inserted into the vacuole and connected through the amplifier to the reference calomel electrode placed in the external medium. During the experiments the cells were kept in a perspex bath with a flowing external solution and were

observed through a horizontal microscope giving magnifications of 100 or 408 times. The block diagram of the apparatus used for potential difference measurements is shown in Fig. 1. Electric potential difference was measured in cells bathed by two types of external medium:

1) containing four kinds of ions (Na^+ , K^+ , Ca^{2+} and Cl^-), denoted as series B,

2) without calcium, denoted as series A. The composition of the solutions in both series is shown in Table 1 and 2.

Table 1

Composition of the solutions of series B (containing Ca^{2+}) (mM)

	Na	K	Ca	Cl
APW	1.0	0.1	0.1	1.3
B1	1.0	0.1	1.0	3.1
B2	1.0	1.0	0.1	2.2

In some cases (experiments of A series) the cells were pretreated with 5 mM NaCl for 24 hours in order to remove exchangeable calcium from the cell wall (H o p e 1961) prior to the experiments.

Table 2

Composition of the solutions of series A
(without Ca^{2+})

Solution	NaCl	KCl
A1	1.05	0.05
A2	1.00	0.10
A3	0.85	0.25
A4	0.70	0.40
A5	0.40	0.70
A6	0.25	0.85
A7	0.10	1.00
A8	1.00	10.00

The electric resistance was measured by the microelectrode technique with the set up shown in Fig. 2. Electric current of ca. $0.5 \mu\text{A}$ is injected into the cell via microelectrode E_{i1} and electrode E_{i2} parallel to the cell. The change in potential difference is recorded by means of microelectrodes E_{v1} and E_{v2} connected to an amplifier or galvanometer. The electric resistance was measured in the cells of *Nitellopsis obtusa* in the following conditions:

Fig. 2. The block diagram of the circuit used for d.c. resistance measurements

Part A passes electric impulses via the pair of electrodes E_{11} , E_{12} . Part B measures the changes in resting potential brought about by the injected current. The pair of electrodes E_{11} , E_{12} is used for the measurement of the voltage. When the distance between the electrode pairs is $1/2$ of the cell length the value of the real d.c. resistance between the vacuole and the medium equals to the measured value (J. Stolarek 1966).

1) in a medium containing Ca^{2+} (APW), 2) in a medium deprived of calcium (the cells were pretreated with 5 mM NaCl before the experiments) to which calcium was added to the medium during the measurements. The results are expressed in $\text{k}\Omega \cdot \text{cm}^2$.

RESULTS

1. *Electric potentials.* The changes in electric potential differences between the vacuole and the medium in cells of *Nitellopsis obtusa*, corresponding to changes in the concentration of potassium and calcium ions in the medium are shown in Table 3. The potential difference was recorded after 15 min. of exposure in the given solution. Some of the cells of this series were pretreated with 5 mM NaCl and they were passed through the solutions A2 (without Ca)-APW-B1-B2. The corresponding changes in electric potential are shown in Fig. 3. The addition of calcium (A2-APW) caused a 54 mV decrease in potential difference. Tenfold increase of potassium concentration accompanied by a tenfold decrease of calcium concentration brought about a decrease in potential difference by 35 mV. When the concentration of calcium was constant in the medium (0.1 mM) but the concentration of potassium decreased from 1.0 to 0.1 mM (B1-B2) the potential difference became more negative by -52 mV.

Another series of potential difference measurements was accomplished on cells pretreated with 5 mM NaCl in order to remove exchangeable calcium from the cell wall. The cells were placed in a bath through which the solutions were flown in the order from A1 to A7 and back. The cell

Fig. 3. The changes in potential difference between the vacuole and external medium in a cell of *Nitellopsis obtusa* corresponding to the sequence of the solution A2 → → APW → B1 → B2

The concentration of Ca^{2+} is tenfold higher in B1 than in APW but in B2 it is tenfold less than in APW, whereas K^{+} concentration in B1 and APW is the same but in B2 it is tenfold weaker. The arrow indicates the moment when new solution was flown in.

Table 3

Changes in electric potential between the vacuole and external medium in cells of *Nitellopsis obtusa*, corresponding to changes of the solutions:

APW—B1, B1—B2, B2—APW

(mV)

Cell	Potential difference measured in			
	APW	B1	B2	APW
1	—102	— 60	— 51	
2	—120	—102	— 63	—123
3	—207	—186	—159	—198
4	—180	—147	— 96	—162
5	—123	— 96	— 54	—117
6	—201	—165	—105	—186
7	—108	— 78	— 48	—102
8	—270	—183	—145	—237
9	—144	—102	— 81	—141
Mean	—161	—124	— 89	—141

$$E_{B1} - E_{APW} = 37 \quad E_{B2} - E_{B1} = 35 \quad E_{APW} - E_{B2} = 52 \text{ (mV)}$$

Table 4

Composition of the solutions of the series B and changes in potential difference corresponding to changes in ionic composition of the medium

Solution	Concentration (mM)				Change in concentration of				Potential diff. change (mV)
	Na	K	Ca	Cl	Na	K	Ca	Cl	
APW	1.0	0.1	0.1	1.3			+10x	+2.3x	+37
B1	1.0	0.1	1.0	3.1		+10x	-10x	-1.4x	+35
B2	1.0	1.0	0.1	2.2		-10x		-1.7x	-52
APW	1.0	0.1	0.1	1.3					

Table 5

Changes in potential difference between vacuole and external medium (mean for 7 cells), corresponding to the passage from solution A2 to A8 and back.
(mV)

A2	A3	A4	A5	A6	A7	A8
-151	-136	-124	-114	-107	-102	-75
-151	-148	-128	-119	-109	-94

Fig. 4. The changes in electric potential difference between the vacuole and external solution of the cell bathed by the solutions of A series (the solutions are changed from A1 to A8)

Table 6

Electric d.c. resistance measured between the vacuole and external medium in cells soaked in APW ($k\Omega$, cm^2)

Cell	Electric resistance measured after insertion of the microelectrode			
	30 min.	1 hour	2 hours	3 hours
1	16.2	16.2		
2	22.2	19.5	22.3	22.3
3	15.8	15.9	16.0	16.0
4	202.8	203.0	203.0	203.0
5	92.0	99.0	92.0	
6	10.0	10.0	10.0	
7	11.0	11.3	11.3	
8	117.0	117.0	117.0	117.0
9	13.1	13.1	13.1	13.1

Table 7

Effect of addition of calcium ions on d.c. resistance between the vacuole and the external solution

Cell (Rca series)	Electric resistance ($k\Omega$, cm^2)		Decrease in resistance corresponding to the passage from A2 to APW (%)
	A2	APW	
1	54	39	27
2	57	20	63
3	46	32	31
4	50	38	33
5	39	25	36
6	25	17	33
7	25	17	34
8	54	36	33
9	94	63	33
10	99	45	55
11	63	42	33
12	30	18	40
13	32	21	34
14	98	63	35
Mean	52	34	37

was bathed for 15 min. by each solution and the potential difference was then recorded. The change in potential difference corresponding to the sequence A2-A7 was -49 mV (Table 5). A further tenfold increase in potassium concentration (A7-A8) from 1.0 to 10.0 mM caused a further

decrease in potential difference which became more positive by 27 mV (Table 5 and Fig. 4).

2. Electric resistance measurements. The results of the control series of resistance measurements made on cells bathed by APW is shown in Tab. 6. It is seen that electric resistance changes little with time (Fig. 5).

Fig. 5. The electric resistance between the vacuole of a cell of *Nitellopsis obtusa* measured first in solution A2 (without Ca^{2+}). The arrow indicates the moment when the solution APW was given

In another series electric resistance was measured between the vacuole and external solution in the medium without calcium (A2) and then in APW (containing Ca^{2+}). The electric resistance decreased very soon by 37% after the addition of calcium (Table 7 and Fig. 5).

DISCUSSION

The results of the measurements of electric potentials in the cells of *Nitellopsis obtusa* showed a dependence of electric potential between the vacuole and the medium on K^+ , Na^+ , C^{2+} and Cl^- concentration in the external solution. The cells kept in the standard APW solution had a steady electric potential difference. However, they fell into two groups: those with potential difference of ca. -134 mV, and another group with p.d. -260 mV. The first value is close to the figure quoted by MacRobbie and Dainty (1958).

In solutions of the series B, the concentrations of K^+ and Ca^{2+} were changed. The sequence APW — B1 corresponding to tenfold increase in Ca^{2+} resulted in a decrease of potential by +37 mV (Tab. 3). When solution B2 replaced B1 (in B2 potassium is ten times more and calcium is ten times less concentrated than in B1) the potential difference became more negative by 35 mV.

It is worth mentioning that the electric potential difference changed when potassium concentration was altered to the same extent in the presence and absence of calcium ions in the external medium. Hope and

Walker (1961) showed that the electric potentials of cells of *Chara australis* responded to a change in external potassium concentration only after pretreatment with 5 mM NaCl and when there was no calcium in the medium. The results obtained in the present work showed that potassium ions have a similar effect on the potential difference in the presence and absence of Ca^{2+} in the medium.

The electric resistance between the vacuole and external solution (APW) for the majority of cells is about $34 \text{ k}\Omega \cdot \text{cm}^2$. This value is about ten times lower as compared to the calculated one. However, in some cases the value of the resistance was as high as $202 \text{ k}\Omega \cdot \text{cm}^2$, i.e. it approached that calculated by MacRobbie and Dainty (1958). In cell depleted of calcium due to pretreatment with 5 mM NaCl and in the case when the measurements were made in Ca^{2+} free media, the resistance was lower than in the solution of the same composition without calcium. When calcium was added to the medium the resistance fell on the average by 37%. The drop in electric resistance brought about by calcium is in disagreement with the data of other authors. It should be noted that the results of our measurements of electric potentials in *Nitellopsis obtusa* do not entirely agree with the data of MacRobbie and Dainty.

For cells with a high potential difference the conclusions of these authors concerning active transport of ions should be altered.

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SUMMARY

Electric potential difference and d.c. resistance between the vacuole and external medium were measured with microelectrode technique in cells of *Nitellopsis obtusa*. A large dispersion in potential difference ranging from -134 to -260 mV was found. The experiments consisting in changing concentration of ions in the medium on electric potentials have shown that potassium and calcium exerted the same effects in cells of this species.

The electric resistance between the vacuole and external medium is in average $34 \text{ k}\Omega \cdot \text{cm}^2$ i.e. it is tenfold less than the theoretical value calculated for this species from ionic fluxes measured with radioactive isotopes. However, in some cells the electric resistance was $202 \text{ k}\Omega \cdot \text{cm}^2$ i.e. it approached the theoretical value calculated by MacRobbie and Dainty. The electric resistance decreased after the addition of calcium ions to the medium, which is in disagreement with Osterhout's data.

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Stosunki jonowe i elektrofizjologia pojedynczych komórek Characee

Cz. I Badania nad potencjałami elektrycznymi i oporem w komórkach *Nitellopsis obtusa*

Streszczenie

Mierzono różnicę potencjałów oraz opór elektryczny dla prądu stałego między wodniczka a środowiskiem w internodialnych komórkach *Nitellopsis obtusa*. Stwierdzono znaczny rozrzut wielkości potencjału elektrycznego od -134 do -260 mV. Obserwacje nad wpływem zmian stężeń jonów w pożywce wykazały, że w przeciwieństwie do innych gatunków ramienic, w tym wypadku jony potasu i wapnia wywierają jednakowy wpływ na potencjał elektryczny komórek tego gatunku. Opór elektryczny między wodniczka a środowiskiem wynosi średnio $34 \text{ k}\Omega \cdot \text{cm}^2$ tzn. jest 10-krotnie niższy w porównaniu z wartością teoretyczną, obliczoną dla tegoż gatunku ze strumieni jonowych zmierzonych za pomocą izotopów promieniotwórczych. W niektórych jednak komórkach opór elektryczny wynosi ok. $200 \text{ k}\Omega \cdot \text{cm}^2$, tj. jest bliski wartości obliczonej przez MacRobbie i Dainty'ego. Opór elektryczny spada po dodaniu do pożywki jonów wapnia, w przeciwieństwie do danych Osterhouta.