ELECTRICAL POTENTIALS AND ITS FLUCTUATIONS IN NODAL CELLS OF *NITELLOPSIS OBTUSA*

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ABSTRACT

Using the liquid microelectrode technique, the electrical potentials of nodal cells and the with them internodal cells neighbouring, of *Nitellopsis obtusa* plants of the *Characeae* family were determined. A specific variation in the potential of nodal cells in the form of cacophony-type irregular disturbances was also found, indicating that the node is not a passive mechanical element controlled by internodal cells, but is a complex system of coupled-, and different metabolic oscillators.

KEY WORDS: *Nitellopsis*, nodal cells, potential changes, biological oscillators.

INTRODUCTION

Investigations of oscillatory variations in the properties of plant cells are mainly conducted on large internodal cells of plants of the *Characeae* family (Nishizaki 1968, Hansen 1978, Toko et al. 1988, Tokarska and Śpiewła 1990, 1994). During the studies of periodic spatial-temporary structures in these cells, it was established, among others, that they do not arise in young plants longer than 2 cm.

Earlier identification of the morphological-functional properties of these plants and, in particular, the structure and properties of nodes (Śpiewła and Skierczyńska 1975, Śpiewła 1982, Śpiewła 1983) led us to investigate whether cells forming nodes (junctions) also show oscillatory behaviour similar to that of internodal cells.

This problem is interesting, because the role of the only mechanical junction coupling internodal cells is very often attributed to the nodes. In our earlier works, we have shown that nodal cells participate in the structural and functional integration very clearly and in a complicated manner. We also found that in the case of division of plants (e.g. as a result of mechanical interactions of the environment), appropriate recovery processes occur in the node, enabling further vegetation of the remaining parts of the plant. The results of work seem to suggest that the functions of nodal cells and of the entire node by exceed far the role of a transfer system and are very complex.

MATERIAL AND METHOD

Measurements were made on the cells of uncorticated species of *Nitellopsis obtusa* (Desv. in Lois) J. Gr. of the *Characeae* family. Plants, collected from the lake Zagłębie, were cultured in the laboratory in aquaria with natural lake

pond water. The concentrations of basic ions in the water were as follows: 0.06 mM K⁺, 0.28 mM Cl⁻, 0.01 mM Mg²⁺ (pH 7.0 ± 0.1). A node with the two internodal adjacent cells were cut from the plant one day prior to the measurements and stored in natural light and temperature conditions (18-19.5°C). Measurements of the electrical potential of the cells were performed using the liquid microelectrode technique, described in our earlier works (Skierczyńska et al. 1972, 1977).

The main difficulty in the measurements was the introduction of microelectrode to the interior (vacuole) of the nodal cell, as shown in Fig. 1 for a young junction of internodal cells of pseudoleaf. Mature nodes of pseudoleaves and pseudostems are very complex because cells of stipels grow from them. Moreover, they have a double, and even triple, layer of irregularly shaped and decomposed nodal cells in the circumferential area (a picture of the pseudoleaf node with two growing stipels is shown in Fig. 2). Hitting by the microelectrode through the opaque circuit layer into the cell of its biconcave plate of the axial region of the node is therefore a matter of chance.

In many experiments it turned out that the tip of the electrode was broken. However, if one succeeds in introducing the microelectrode to the vacuole of a nodal cell, then the fact of successful insertion may be achieved by withdrawing the microelectrode very slowly and by observing a sudden decrease in the potential during the exit of the electrode tip from the cell cytoplasm. The successful insertion of the microelectrode may thus be evaluated from the value of the potential, which is comparable with that one measured by the microelectrode inserted in the internodal cell.

In many cases, nodal cells undergo so significant a damage during the microelectrode introduction that they lose turgor and decay. (The microelectrodes used had a tip diameter of 2-

Fig. 1. Schematic illustration of lateral and longitudinal section of a *Nitellopsis obtusa* nodal complex of a pseudoleaf. The symbols are: m-el – liquid microelectrodes with 3 MKCl, Ag/AgCl – silver silver chloride electrode.

Fig. 2. General view of a mature node of a pseudoleaf of *Nitellopsis obtusa* (optical micrograph).

3 μm ; and at distances of 20–25 μm from their tip, the microelectrode diameters approached 10 μm while the diameters of the studied cells were 20–60 μm). In these situations, the potential measured between the microelectrode localised in the damaged cell and the reference electrode becomes very unstable, and suddenly decreases in the first few seconds of the observation. Then it decreases slowly but continuously during a few to several minutes. The microelectrode was inserted into the vacuole of one of the adjacent cells by the node- of internodal cells. Finally, after obtaining the signal of stable or oscillating potential, a microelectrode was inserted in the node.

In order to facilitate the understanding of the experimentally obtained results and their discussion, it should be mentioned that nodal cells are interconnected between themselves and with the internodal cells by a strongly developed system of plasmodesmata. According to earlier papers (Śpiewła 1982, 1987), plasmodesmata are canals penetrating walls of intercellular contacts (diameters of 50–70 nm), strewn by plasmalemma and penetrated by endoplasmic reticulum tubules. In the case of *Nitellopsis obtusa*, the surface of the walls occupied by holes of plasmodesmata is about 1% and 0.7% in the contact of nodal and internodal cells of pseudostem and in the contact of node-node cells, respectively.

An example of the plasmodesmata longitudinal sections in the wall between internodal and nodal cells of pseudostem is shown in Fig. 3.

RESULTS

The data obtained are average values from a series of measurements made for 16 nodes. For the first time, a direct com-

parison of the values of the resting potentials of nodal and internodal cells, localised beneath the investigated node, is made (Table 1). Each value of the potential, listed in Table 1, is the arithmetic mean of at least five measurements made within 40 min counted from the moment of introduction of the microelectrode. The data presented here refer to the situations when the internodal cells did not reveal oscillations in the resting potentials.

In five cases, the potential, measured in the internodal cells localised beneath the studied nodes, changed periodically. In two of the cells, the oscillations has a period of about 40 min, in the other two the period was about 13 min, while in the fifth cell it was about 27 min. The latter two cells of the oscillation period of about 13 min, both cells revealed simultaneously changes in the potential with a period of several tenth of seconds. The average value of the amplitude of changes in the potential of the five cells was 5.7 mV with a scatter in these changes in different cells within in the range 2.5–9.5 mV.

By recording the resting potential in nodal cells for relatively long periods (even up to 1.5 h), it was found that the oscillations are not generated in these cells, unlike in the case of internodal cells. In 13 out of 16 cells studied however, a specific variation in the potential, of cacophony-type irregular disturbances, were observed. An example of the registration of these changes for cell no. 7 is presented in Fig. 4. The amplitude of these irregular changes reached even 90% of the average value of the resting potential (see Table 1). In an experimental way, it is excluded that the observed impulses and disturbances are equipment artefacts or are connected with the instability in the functioning of electrodes and microelectrodes.

TABLE 1. Average values of resting potentials of nodal and internodal cells *Nitellopsis obtusa*.

Cell no.	Nodal cells (mV)	Internodal cells (mV)	Differences in resting potentials (mV)
1.	106.0	104.0	+ 2.0
2.	85.5	85.5	+ 0.5
3.	120.0	119.5	+ 0.5
4.	90.0	87.0	+ 3.0
5.	114.5	115.0	- 0.5
6.	70.0	67.5	+ 2.5
7.	98.5	98.0	+ 0.5
8.	142.0	141.5	+ 0.5
9.	108.5	109.0	- 0.5
10.	79.0	76.0	+ 3.0
11.	103.0	103.0	0.0

Mean value = +1.4 mV
(calculated without serial no. 5 and 9)

DISCUSSION

The obtained results of measurements of resting potential lend support for earlier observations (Śpiewła 1979) that the potential of successive cells, counting from the plant summit, are smaller and smaller (in terms of absolute values) and this is also true in the case of nodal cells. From the data presented above, it follows that the values of resting potentials of nodal cells indirectly determine the values of potentials of the neighbouring internodal cells through the node.

The results of fluctuating changes in the resting potential, observed in a majority of the investigated nodal cells, seem decidedly more surprising. In view of the character of plasmodesmatic coupling between cells, it may be expected that oscillatory changes in the potential of internodal cells should force rhythmic changes in nodal cells. In contrast to this, the registration of potential disturbances of these cells indicate that they are arrhythmic.

The fact, corroborated earlier, that periodic spatial-temporary structures do not develop in cells of length smaller than 2

cm (Śpiewła and Tokarska 1990) seems to be a sufficient indication to believe that such structures do not form in nodal cells. This conclusion agrees with the sufficiently documented hypothesis (Tokarska and Śpiewła 1993, 1994) that synchronic changes in the open and closed plasmodesmata are most probably responsible for oscillatory changes in the electrical conductance of nodes (Lyalin et al. 1986, Tokarska and Śpiewła 1993, 1994).

Explanation of arrhythmic changes in the potential in nodal cells becomes possible if it is assumed that the node is not a passive element determined by the properties of internodal cells but is composed of a complex system of coupled and different oscillators. Since the behaviour of a set of oscillators on different frequencies depends on coupling forces, it may be expected, and has been proved mathematically, that for poor interactions the oscillators do not reach synchronisation and the set as a whole becomes a source of cacophony even when all the oscillators are set in motion simultaneously. It has been demonstrated (Winfree 1987) that the behaviour of such systems depends on the range of frequency distribution.

Fig. 3. Longitudinal section of a nodal cell and internodal cell wall junction of a pseudostem node (fixed in potassium permanganate at pH 7.0).

Fig. 4. Changes in the electrical potential of nodal cell no. 7 (see table 1).

If the differences are significant in comparison with the magnitude of coupling forces, the system also tends to a chaotic behaviour; but if the differences decrease to a particular critical value, a part of the system may spontaneously attain, for a short time, a synchronic state (Strogatz et al. 1992). Thus, even a system apparently of symmetrically coupled oscillators, as a node seems to be, can show irregular variation, as recorded in our experiments.

The validity of the above interpretation means that oscillations in the potential occurring in particular nodal cells, have the character of metabolic changes rather than membranous ones (Berridge and Rapp 1979). So far there is a lack of systematic investigations dealing with metabolic oscillations in *Characeae*. However, studies of ultraweak luminescence, presently being carried out in our laboratory, of cells of these plants in situations of their exposure to different biologically-active substances provide more and more evidence of their existence.

LITERATURE CITED

- BERRIDGE M. J., RAPP P. E., 1979. A comparative survey of the function, mechanism and control of cellular oscillators. *J. Exp. Biol.* 81: 217-279.
- HANSEN U.-P., 1978. Do light induced changes in the membrane potential of *Nitella* reflect the feed-back regulation of a cytoplasmic parameter? *J. Membr. Biol.* 41: 197-224.
- LYALIN O. O., KTTOROWA I. N., BARMICHEVA E. M., AKHMEDOV N. I., 1986. Intercellular connections in submersed trichomes of *Salvinia*. *Fisiol. Rast. (Moscow)* 33: 432-446.
- NISHIZAKI Y., 1968. Rhythmic changes in the resting potential of single plant cell. *Plant Cell Physiol.* 9: 613-616.
- SKIERCZYŃSKA J., ŚPIEWŁA E., BULANDA W., PRZYGODZKA A., 1972. Measurements of membrane resistance of *Characeae* with external electrodes and microelectrodes. *J. Exp. Bot.* 23: 591-599.
- SKIERCZYŃSKA J., ZARĘBSKI W., SIELEWIESIUK J., ŚPIEWŁA E., 1977. Some methods for the measurements of tonoplast resistance of *Nitellopsis obtusa*. *J. Exp. Bot.* 102: 37-55.
- ŚPIEWŁA E., 1982. The kinetics of after-injury processes in the nodes of *Characeae*. *Folia Soc. Sci. Lubl.* 24: 19-27.
- ŚPIEWŁA E., 1983. "Elektrofizjologiczne aspekty wpływu niektórych czynników środowiska na rośliny". Wyd. Pol. Lub. Lublin, ss. 138.
- ŚPIEWŁA E., 1987. Properties, structure and some physiological functions of *Characeae* nodes. *Prace Nauk. Pol. Lub. Lublin, Fizyka* 5: 53-71.
- ŚPIEWŁA E., SKIERCZYŃSKA J., 1975. Elektryczny układ zastępczy węzłów roślin *Characeae*. *Folia Soc. Sci. Lubl.* 17: 147-155.
- ŚPIEWŁA E., MATUSIEWICZ J., 1982. The structure and healing processes of plasmodesmata in *Characeae*. *Folia Soc. Sci. Lubl.* 34: 29-33.
- ŚPIEWŁA E., TOKARSKA M., 1990. Oscillations of membrane potential during inhibition of cytoplasmic streaming in *Characean* internodes. *Physiol. Plant.* 80: 191-195.
- TOKO K., HAYASHI K., YOSHIDA T., FUJIYOSHI T., YAMAFUJI K., 1988. Oscillations of electric spatial patterns emerging from the homogenous state in *Characean* cells. *Eur. Biophys. J.* 16: 11-21.
- TOKARSKA M., ŚPIEWŁA E., 1993. Oscylacyjne zmiany przewodnictwa membranowego i plazmodesmatycznego złącz komórek *Nitellopsis obtusa*. *Prace Nauk. Pol. Lub., Fizyka* 9: 103-108.
- TOKARSKA M., ŚPIEWŁA E., 1994. Oscillations in the electrical resistance of *Nitellopsis obtusa* nodes. *Physiol. Plant.* 90: 98-104.

POTENCJAŁY ELEKTRYCZNE KOMÓREK WĘZŁOWYCH
NITELLOPSIS OBTUSA I ICH FLUKTUACJE

STRESZCZENIE

Wykorzystując technikę mikroelektrod cieczowych określono potencjały elektryczne komórek węzłowych i sąsiadujących z nimi komórek międzywęzłowych roślin *Nitellopsis obtusa* z rodziny *Characeae*. Wykryto również specyficzną zmienność potencjału komórek węzłowych o charakterze nieregularnych zaburzeń typu „kakofonii”, co świadczy, że węzeł nie jest biernym, mechanicznym elementem kontrolowanym przez komórki międzywęzłowe, lecz stanowi złożony układ sprzężonych i różnorodnych oscylatorów metabolicznych.

SŁOWA KLUCZOWE: *Nitellopsis*, komórki węzłowe, zmiany potencjału, oscylatory biologiczne.