Biochimica et Biophysica Acta, 554 (1979) 62—67

BBA 78391

THE EFFECT OF CELL EXCISION AND MICROELECTRODE PERFORATION ON MEMBRANE RESISTANCE MEASUREMENTS OF *NITELLA TRANSLUCENS*

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(Received July 17th, 1978)
(Revised manuscript received December 18th, 1978)

Key words: Membrane resistance; Cell excision; Microelectrode perforation; Leakage; (Nitella translucens)

Summary

The effect of cell excision and microelectrode perforation on the membrane resistance measurements of *Nitella translucens* was determined by direct experiment. From the results it is concluded that perforation has no effect on cells as short as 1 cm. Current leakage though the node of an excised cell has however to be given some consideration. The method used for determining the resistance recovery to insertion has a wide application and its simplicity will encourage its use in other biological systems.

Introduction

It has been generally assumed that the damage produced to the membrane by the microelectrode perforation is minimal and that after recovery the current leakage occurring through the electrode perforation is neglegible. In studies of the *Characeae* it has also been assumed that current leakage though the nodes of an excised cell is neglegible. The validity of both assumptions has been investigate but until now the evidence seems contradictory.

The effect of electrode perforation was studied by Spanswick [1] who observed that the membrane resistance of *Nitella translucens* reaches a steady value few hours after electrode insertion. He concluded that after this recovery period the membrane resistance is faithfully determined. On the other hand Tazawa et al. [2] compared the membrane resistance values of *Nitella flexilis*

Abbreviation: MES, 2-(*N*-morpholino)ethanesulfonic acid.

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measured with and without electrode insertion and concluded that electrode perforation leads to a substantial underestimation of the membrane resistance. This seems to indicate that in spite of the observed recovery, the cell membrane is irreversibly damaged by electrode insertion.

Similar estimations of the leakage through the microelectrode perforation has been done for slow muscle fibres by Stefani and Steinbach [8], who found that it leads to an underestimation of both membrane resistance and potential. Hodgkin and Nakagima [9] found similar values for the shunt resistance appearing in skeletal muscle fibres but they estimated a smaller correction for membrane resistance values.

The possible current leakage through the cell nodes is usually disegarded by the assumption that the nodes act as infinite impedance terminations [3]. This was apparently confirmed by Hogg et al. [4] who found that a cable model for the *Characeae* based on the last assumption reproduces remarkably well the observed cell response to a D.C. pulse. However direct measurements of the nodal resistance between two adjacent cells [5—7] indicates that this resistance is by no means infinite. However all these measurements were performed on nodes with intact cells on both sides, and it might be expected that the nodal resistance would increase substantially when one of the cells is removed. This premise is directly tested in the present work. It is shown that there is indeed a nodal resistance increment, but that some care is required to make the assumption of infinite impedance terminations.

Usually, estimates of the leakage though the perforation have being done by indirect measurement, assuming that the potential recovery, or lack of recovery, indicates the magnitude of the leakage. This assumption could however not be valid and it is thus convenient to be able to determine in a direct manner if the measured resistance reproduces the resistance value prior to electrode insertion. This is done in the present work for *Nitella translucens*. The experimental technique has however wider application and its simplicity will encourage its use in other biological systems.

Material and Methods

Nitella translucens plants were originally collected from a loch near Dunkeld, Scotland, and transferred to shaded tanks out-of-doors. The plants were rooted in a sand and soil mixture and they grew vigorously after acclimatization. The cells were watered with artificial pond water of the following composition: 0.1 mM KCl, 0.1 mM $CaCl_2$ and 1 mM NaCl. The pH of the artificial pond water was adjusted to pH 5 by the addition of HCl and buffered with 2-(*N*-morpholino)ethanesulfonic acid (MES).

The cell bath consisted of a three compartment vessel made from perspex (Fig. 1). The separation between the compartments was achieved with perspex barriers. Along the center of this barrier runs a 1 mm groove into which the cell is mounted. It was found that good electrical insulation is obtained if a silicone compound (MS4, Dow Corning) is used for smearing the groove, instead of white vaseline, and silicone rubber (Blue-Tack, Bostik) is used for smearing the perspex barriers. The illumination, 0.04 MW/cm^2, was provided by a tungsten

lamp and heating of the cell bath reduced to a minimum by a combination of glass, air and perspex filters.

The current injecting electrodes consisted of two connected pairs of Ag/AgCl wires located in the side compartments and another pair located in the central compartment. The voltage recording electrodes to be inserted into the cell consisted of glass micropipettes filled with a 3 M KCl solution by the microfibre method. The reference electrode, consisted of a glass tube (1 mm outer, 0.5 mm inner diameter) which had been heated at one end to give a small orifice (≈ 50 μm) and then filled, by boiling, with 3 KCl-agar solution. The electrodes were connected to stabilized calomel half cells by means of KCl-agar bridges and then to the usual electronics.

Results

Cell recovery after microelectrode insertion

The recovery of the membrane resistance after electrode perforation as a function of time can be measured by doing the following experiment which uses the experimental arrangement described in Fig. 1.

From Fig. 1 it can be seen that, when a current pulse is applied between electrodes A and C the resulting voltage response is given by

$$V_{12} = I(R_A + R_C)$$

where R_A and R_C are the resistances of the cell segments A and C and I is the applied current. If electrode 3 is inserted into the cell and the same current I applied, the voltage responses between electrodes 1 and 2 (V'_{12}) and electrodes 1 and 3 (V'_{13}) are given by:

$$V'_{12} = I(R_A + R'_C) \quad \text{and} \quad V'_{13} = IR_A$$

where ($'$) denotes the value after insertion. The ratio between the membrane resistance of the central segment before and after perforation can thus be obtained from:

$$V'_{12}/V_{12} = (R'_C + R_A)/(R_C + R_A)$$

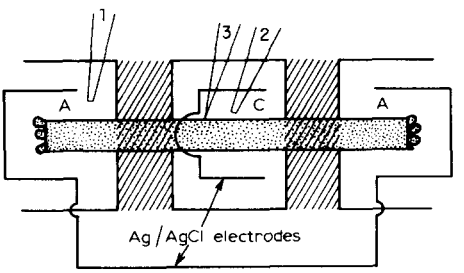

Fig. 1. Experimental arrangement used for the measurement of the resistance recovery after microelectrode insertion. The shaded areas represent insulating barriers. 'A' represents the lateral segments and the corresponding membrane area in these compartments. 'C' represents the central compartment and the corresponding membrane area. Current was applied through the Ag/AgCl wires and the response recorded by electrodes 1, 2, and 3.

TABLE I

THE RECOVERY OF THE MEMBRANE POTENTIAL E(mV) AND THE MEMBRANE RESISTANCE R_m (k$\Omega \cdot cm^2$)

Number of cells = 16.

Time (min):	1		5		15		30		60	
	R_c/R'_c	E	R_c/R'_c	E	R_c/R'_c	E	R_c/R'_c	E	R_c/R'_c	E
Av.	0.53	81	0.55	86	0.76	99	0.85	104	0.98	109
S.D.	0.24	11	0.21	15	0.23	13	0.20	13	0.11	11

Since

$$R_C/R_A = V_{12} - V'_{13}/V'_{13}$$

then

$$\frac{R'_C}{R_C} = \frac{V'_{12} - V'_{13}}{V_{12} - V'_{13}}$$

This ratio was computed for different times after the electrode insertion and the collected results are presented in Table I. It can be seen that the membrane resistance had recovered after 1 h to its value prior to insertion. It should, however, be mentioned that in some cases the membrane potential did not follow the recovery pattern of the membrane resistance, thus indicating a possible weakness of the usual way of estimating the leakage.

Similar recovery periods have been reported [10]. Longer periods (4 h) were reported by Spanswick [1] for experiments carried out in the dark, where the membrane resistance is as much as five times bigger than its value in the light and thus it is to be expected that recovery to these very high resistance values can only be achieved over more extended periods.

Direct measurements of the nodal resistance between two adjacent cells, before and after excision of the neighbouring cell, appear to be a relatively simple matter. This was done using the arrangement shown in Fig. 2 where the

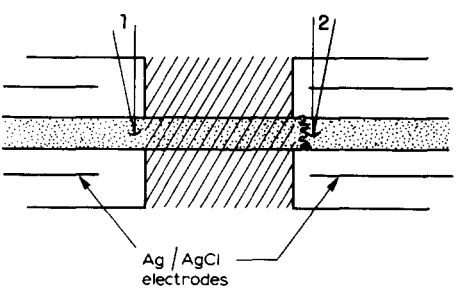

Fig. 2. Experimental arrangement used for the measurement of the transnodal resistance and its response to cell excision. The shaded areas represent an insulating barrier and the squiggly line the cell node. Current was applied through the Ag/AgCl electrodes and the transnodal potential recorded by microelectrodes 1 and 2.

TABLE II

THE RESISTANCE ($k\Omega \cdot cm^2$) OF THE CELL NODE AS A FUNCTION OF TIME AFTER EXCISION OF ONE OF THE CELLS

Number of cells = 13.

Time:	0	10 min	1 h	2 h	3 h	4 h
Average	1.30	1.80	2.13	2.36	2.45	2.58
S.D.	0.57	0.88	0.89	0.82	0.86	0.95

potential across the node is recorded by electrodes 1 and 2. When current pulses (0.05 A/cm^2) were applied by electrodes A and B the potential response of the node gave a direct recording of the transnodal resistance. Current pulses were applied at regular intervals. The collected results are reported in Table II. Here, it is shown that, over a period of a few hours, the average increase in the transnodal resistance is more than 2-fold, though the actual increase varies greatly from cell to cell. It should also be mentioned that the transnodal resistance values obtained for two unexcised cells agrees with those reported by Spanswick [1] for the same species.

Discussion

In the present work it has been shown that the membrane resistance of *Nitella translucens* recovers to its value prior to electrode insertion. This disagrees with the finding of Tazawa et al. [2] that suggest the irreversible damage of the membrane. In their experiments they compared the resistance measured by the open vacuole and microelectrode techniques and certainly these comparisons indicate that larger resistances are obtained with the former method. However these comparisons may not be entirely valid since the open-vacuole technique requires the presence of osmotic agents. Furthermore they fail to mention if the same light regimes were used in the two types of experiments. The dependence of the membrane resistance on light intensity [12] could account for the observed difference. It should be noted that cell recovery depends ultimately on the amount of damage caused by electrode insertion. In the present work the criteria of a good insertion were that it did not produce migration of the chloroplasts away from the electrode tip or stop the cytoplasmic streaming for more than 1 min. From the results obtained here it can be concluded that cell segments as short as 1 cm do not show any effect of electrode perforation; of course, for very short segment this might not be valid: i.e. as the ratio of injured area to total area increases.

In this work it is also shown that the nodal resistance increases as a consequence of excising the neighbouring cells. Even allowing for the average two-fold increase, the value of the transnodal resistance, which is the measured ohmic resistance multiplied by the node area, is still small compared with the membrane resistance. However, in making the assumption of infinite impedance for the nodes it is the total resistance of the membrane relative to the total resistance of the node which is important. In the case of a cell of 8 cm length and 1 mm diameter the total resistance of the node is in fact sixteen times

bigger than the total resistance of the membrane so that only 1/16 of the current flows through the node. The assumption of infinite impedance is quite sound in this case but it will not be valid for short cells and the techniques based on this assumption [3,13] will not apply. Of course in the studies of isolated cell segments this limitation does not arise because the infinite impedance terminations are insured by means of the isolating barriers.

It seems natural to extend the conclusions of the present work to other characean species, but the experiments are so simple as to encourage their repetition for any long cylindrical cell. This would be particularly convenient before performing flux experiments simultaneous to membrane potential recording in very short cells, were the current leakage could be critical.

Acknowledgements

I am very grateful to J. Hogg and E.J. Williams for their help and encouragement. This work was supported in part by CONACyT, Mexico.

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