

Short Communication

Higher Plant Cell Membrane Resistance by a Single Intracellular Electrode Method¹

Received for publication May 21, 1973 and in revised form September 24, 1973

W. P. ANDERSON,² D. L. HENDRIX,³ AND N. HIGINBOTHAM

Department of Botany, Washington State University, Pullman, Washington 99163

A single intracellular microelectrode technique has been adapted to measure membrane resistance in a higher plant cell. As a direct result of the convenience of this method, which allows relatively long term recordings on a single cell, it has been found that membrane resistance increases for about 30 minutes after cell impalement in *Pisum sativum* L. cv. Alaska root cortical cells, although cell potential is established at a constant value in less than 2 minutes. It is proposed that these observations imply a regulating feedback loop between electrogenic pump rates and membrane potential.

Previously reported measurements of higher plant cell membrane resistance (3, 4, 17) have been made by inserting two microelectrodes into a cell, one to inject current and the other to record potential difference. With large coenocytic algae, this can be achieved in a fairly routine way, but in higher plant cells which may measure no more than 20 μm in diameter by 80 μm long, it is not easy to insert two microelectrodes into a single cell so that the membrane seals around both and a "normal" cell PD⁴ is recorded. Single electrode resistance measurements using the balanced-bridge technique (1) have not been used much in plant cell studies. It is, in certain respects, an unattractive method which only indirectly distinguishes the electrode tip resistance from the membrane resistance; accurate measurement of membrane resistance requires no alteration in electrode resistance upon cell impalement, a requirement more likely to be realized in animal cells because of the absence of a cell wall. We describe here the application to plant cells of a single intracellular microelectrode method of membrane-resistance measurement and give sample results obtained on root cortical cells of *Pisum sativum* cv. Alaska.

The technique used is basically that described by Brennecke and Lindemann (2) to which the reader should refer for details of the underlying theory. It was not found necessary to employ

the operational amplifier circuit given by these authors to provide a high impedance current source. Unipolar square-wave signals were fed to the impaling electrode through a $10^9 \Omega$ resistor (Victoreen Hi-Meg) from an Exact 500B waveform generator (Fig. 1). In all other respects, the measurement system is similar to that described by Brennecke and Lindemann (2). Briefly, the underlying idea of this method is that one can discriminate the membrane resistance, which is shunted by a membrane capacitance of about 100 picofarads in a typical higher plant cell of surface area 10^{-4} cm^2 , from the electrode resistance which is shunted only by a small stray capacitance; the time constant of the membrane RC network is approximately 1 msec, while that of the electrode network is of the order of 10 μsec . The technique relies on the electrode RC network being a negligibly poor voltage store at the frequency of pulsing (and so causing zero displacement of the resting PD), while the membrane RC network becomes charged and displaces the cell PD from its resting value. The considerations governing choice of pulsing frequency can be found in the original article (2).

We made our measurements by injecting a train of unipolar (positive or negative) square-wave pulses of frequency 10 kHz and pulse height 1 to 5 namp. The resulting voltage signals from the cell were displayed on one trace of a Tektronix Model 532 oscilloscope fitted with a type 1A1 dual trace unit coupled through a Bioelectric NF1 recording amplifier, input impedance $10^6 \Omega$ (Fig. 1). The second oscilloscope channel was connected directly to output II of the wave generator, set to give signals identical to those from output I which were applied to the Hi-Meg resistor. From these voltage measurements, the current injected into the cell could be determined from a calibration curve. The intracellular electrodes were drawn from glass capillary tubes and filled with 3 M KCl; those with tip resistances in the range 3 to 15 megohms were selected for use. The reference electrode/external current electrode was also a glass micropipette, approximately 50 μm tip, filled with 3 M KCl in 2% agar. Ag/AgCl wire electrodes coupled these capillary salt bridges to the measuring circuit.

The experimental sequence used was as follows. An intact seedling of *Pisum sativum* cv. Alaska, grown for 4 days after germination in aerated liquid culture of 1X solution, 1 mM KCl; 1 mM $\text{Ca}(\text{NO}_3)_2$; 0.25 mM MgSO_4 ; 0.904 mM NaH_2PO_4 ; 0.048 mM Na_2HPO_4 (8) in the light at 20 C, was mounted in a Mertz chamber (7) containing 1X solution. While the microelectrode was still in the solution, the tip potential (no more than several millivolts) was recorded and offset on the oscilloscope either by adjusting the balance on the NF1 amplifier or by adjusting the vertical control on the oscilloscope. A train of pulses was then applied and made square by adjusting, if

¹ This research was supported by National Science Foundation Grant GB 19201 and in part by funds provided for biological and medical research by the State of Washington Measure No. 171.

² Permanent address: Department of Botany, University of Liverpool, Liverpool, England.

³ Present address: Department of Botany and Plant Pathology, Purdue University, Lafayette, Ind. 47907.

⁴ Abbreviation: PD: potential difference.

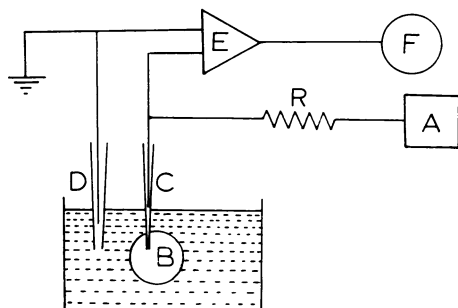


FIG. 1. Block diagram of measuring circuit. A: waveform generator; R: Victoreen Hi-Meg resistor ($10^9 \Omega$); B: cell; C and D: intracellular and external electrodes, respectively; E: recording amplifier; F: oscilloscope.

necessary, the input capacitance compensator of the NF1 amplifier. No displacement of the trace baseline was found with the electrodes in solution for either positive or negative pulse trains, provided that the pulses were square and that the electrodes were unpolarized. No change in tip potential due to current flow was ever noticed. The duration of a pulse train was usually less than 10 sec; the polarity of the pulse train was alternated from run to run (this prolonged the usefulness of the Ag/AgCl electrodes). A root cortical cell was then impaled and the resting potential recorded. As is generally observed, the membrane potential rapidly stabilized at the resting value (Fig. 3). Cell PD values less than -110 mV in this particular preparation were thought atypically low and such impalements were not used; measurement of resistance on these cells did in fact give very low values and the implication is that there was incomplete membrane sealing after electrode penetration in these cases.

Membrane resistance is obtained from measuring the displacement of the square-wave baseline from the original (zero current) cell-potential level (Fig. 2). The displacement in millivolts is equal to $RI/2$ where R is the membrane resistance (megohm) and I is the pulse height (nanoamp) for a pulse train with a pulse-period ratio of 0.5. The best estimate of membrane resistance at any given time was taken as the mean of the values found with positive and negative current pulses. This helped in recording resistances while membrane potential was rapidly changing (as in the 1st min in Fig. 3); the total displacement of the trace baseline in switching from positive to negative pulses was used (and is, of course, equal to RI). This is apparently acceptable because after the cell PD had stabilized, it was confirmed on many trials that the magnitude of the PD change induced by positive pulses is insignificantly different from that caused by equal but negative pulses; thus no rectification was observed.

Three factors should be examined to ensure the validity of the measurements: (a) electrode resistance changes on cell impalement; (b) electrode tip potential changes on impalement and with current passage; (c) electrode polarization with current passage. The first of these causes indirect problems only; the method successfully discriminates the electrode resistance and, indeed, allows it to be continuously monitored since the peak-to-peak amplitude of the square-wave output from the cell in millivolts is given by $R_E I$ where R_E is the electrode resistance (megohm). Our observations indicate that R_E usually increases by several megohms on the first impalement, but thereafter remains relatively constant for several successive impalements; the indirect problem is that instability may be produced if $R_E > 20$ megohms. No evidence has been found for current-induced tip-potential changes with both

electrodes in solution either before or after cell impalement. No direct evidence is available for such an effect with the electrode implanted, but every single electrode technique suffers this shortcoming, and the ohmic nature of the response of membrane resistance, as measured, strongly suggests that we are not observing a current-induced tip PD. The third factor, electrode polarization, causes serious difficulties; the response to current flow is indistinguishable from the membrane-resistance response, *i.e.* a displacement of the baseline of the square-wave signal. However, it is easily screened out by the experimental sequence described earlier. Provided that there is no displacement of the trace baseline with both electrodes in solution before and after impalement, one can be confident that the electrodes are not polarized and that the resistance measurements have not been affected. The Ag/AgCl wires were cleaned and plated at regular intervals (after approximately 2 min of cumulative current flow) to further minimize the possibility of polarization effects.

Finally, we wish to comment briefly on the results we have obtained with this technique; a more detailed report will be given later. Figure 3 shows the variation with time, from impalement, of the cell PD and membrane resistance of a first-layer cortical cell at about 1 cm from the root tip. This cortical cell had a surface area of $1.4 \times 10^{-4} \text{ cm}^2$ so that the maximum membrane resistivity of the plasmalemma-tonoplast combination, assuming no current flow through the plasmodesmata, was $1.8 \text{ k}\Omega \text{ cm}^2$. The mean resistivity from eight cells was $2.1 \text{ k}\Omega$

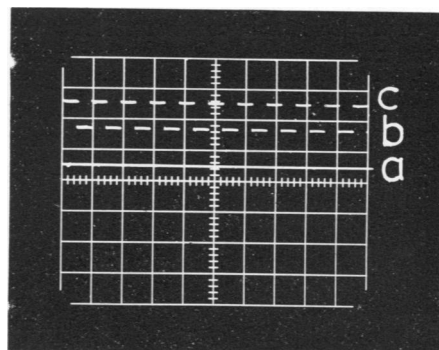


FIG. 2. Sample oscilloscope trace. A: cell resting PD; B: baseline of the cell response to an injected hyperpolarizing square-wave current (10 kHz). The distance $AB = IR/2$, and the pulse height $BC = IR_E$.

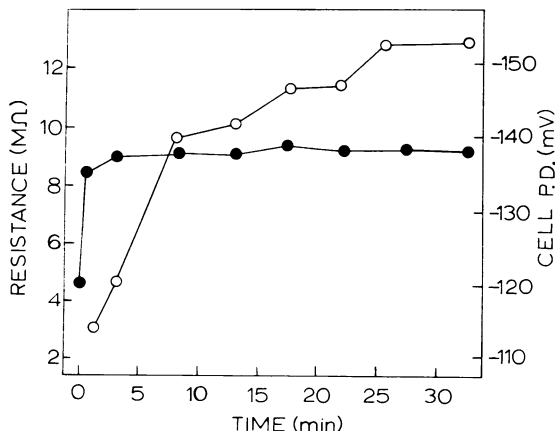


FIG. 3. Graph of total cell resistance (O) and cell resting PD (●) versus time elapsed from impalement for a *Pisum sativum* root cortical cell.

cm², and the resistance was ohmic for currents from 1 to 5 namp, positive and negative. Note that the long term resistance increase bears no relation to the cell PD which remains essentially constant after the 1st min from impalement. A similar resistance increase has been reported in the algae (9, 10, 12), although the interpretation is still in dispute (10, 12). This is, to our knowledge, the first explicit report of increase in total cell resistance with elapsed time from cell impalement in higher plant tissue.

The observation of relatively constant cell PD during a long term increase in membrane resistance prompts a speculative comment. Electrogenic ion pumps have been suggested in explanation of data from algae (5, 13), fungi (11), and higher plants (4); in the latter some 60% of the observed PD is thought to be due to electrogenic pump action. Following Kitasato (6) we write

$$E_M = E_G + iR$$

where E_M is the observed membrane potential, E_G is the potential from the Goldman diffusion equation, i is the current through the electrogenic pump and R is the membrane resistance. Assume that electrode penetration causes membrane injury so that E_G , R , and possibly i decrease. Recovery of E_M may be achieved by increases in E_G , i , or R . Figure 3 shows that E_M recovers more rapidly than R and E_G (the decrease in E_G , for a successful cell impalement, will be due primarily to a partial short circuiting of the ion gradients through the low resistance pathway of the damaged membrane, rather than to massive loss of the cell ionic contents). Thus, the only variable which can cause recovery of E_M more rapidly than recovery of R is the electrogenic pump current i . We therefore suggest that there is a regulatory feedback loop, of mechanism at present unspecified, between the cell PD and the electrogenic pump rate; the initial decrease in cell PD upon impale-

ment stimulates the pump to work faster (which may in itself cause a reduction in measured resistance, since the pump will carry current) and so increase i to stabilize E_M . As membrane repair proceeds and E_G and R increase, the pump rate apparently declines (i decreases) so that E_M remains constant.

LITERATURE CITED

1. ARAKI, T. AND T. OTANI. 1955. Response of single motoneurons to direct stimulation in toad's spinal cord. *J. Neurophysiol.* 18: 472-485.
2. BRENNER, R. AND B. LINDEMANN. 1971. A chopped-current clamp for current injection and recording of membrane polarization with single electrodes of changing resistance. *T. I. T. J. Life Sci.* 1: 53-58.
3. GREENHAM, C. G. 1966. The relative electrical resistances of the plasmalemma and tonoplast in higher plants. *Planta* 69: 150-157.
4. HIGINBOTHAM, N., J. S. GRAVES, AND R. F. DAVIS. 1970. Evidence for an electrogenic ion transport pump in cells of higher plants. *J. Memb. Biol.* 3: 210-222.
5. HIGINBOTHAM, N., A. B. HOPE, AND G. P. FINDLAY. 1964. Electrical resistance of cell membranes of *Avena* coleoptiles. *Science* 143: 1448-1449.
6. KITASATO, H. 1968. The influence of H⁺ on the membrane potential and ion fluxes of *Nitella*. *J. Gen. Physiol.* 52: 60-87.
7. MERTZ, S. M., JR. 1973. Electrical potentials and kinetics of potassium, sodium, and chloride uptake in barley roots. PhD dissertation, Washington State University, Pullman.
8. PIERCE, W. S. AND N. HIGINBOTHAM. 1970. Compartments and fluxes of K⁺, Na⁺ and Cl⁻ in *Avena* coleoptile cells. *Plant Physiol.* 46: 666-673.
9. SKIERCZYŃSKA, J. 1968. Some of the electrical characteristics of the cell membrane of *Chara australis*. *J. Exp. Bot.* 19: 389-406.
10. SKIERCZYŃSKA, J., R. ŻOLNIERCZUK, E. ŚPIEWLA, W. BULANDA, AND A. PRZYGOZDZKA. 1972. Measurement of membrane resistance of Characeae with external electrodes and microelectrodes. *J. Exp. Bot.* 23: 591-599.
11. SLAYMAN, C. L. 1965. Electrical properties of *Neurospora crassa*: Respiration and the intracellular potential. *J. Gen. Physiol.* 49: 93-116.
12. SPANSWICK, R. M. 1970. Electrophysiological techniques and the magnitudes of the membrane potentials and resistances of *Nitella translucens*. *J. Exp. Bot.* 21: 617-627.
13. SPANSWICK, R. M. 1972. Evidence for an electrogenic ion pump in *Nitella translucens* I. The effects of pH, K⁺, Na⁺, light and temperature on the membrane potential and resistance. *Biochim. Biophys. Acta* 288: 73-89.
14. SPANSWICK, R. M. 1972. Electrical coupling between cells of higher plants: a direct demonstration of intracellular communication. *Planta* 102: 215-227.