6 Use of Double Barrel Micropipettes to Voltage-Clamp Plant and Fungal Cells

ROGER R. LEW

6.1 Intracellular measurements in intact, turgid cell compared with protoplasts

In a paper published in 1977, Racusen et al. documented a startling property of higher plant cells: The membrane potential of a higher plant cell responds to the extracellular osmolarity, depolarizing under hyper-osmotic treatment, up to and including the point of plasmolysis. The effect was reversed by removing the extracellular osmoticum. Isolated protoplasts exhibited very depolarized potentials compared with intact turgid cells. Osmotic effects on the electrical properties extend beyond changes in the membrane potential. The conductance, a measure of the voltage dependence of ionic current flow across the plasma membrane, also changes in response to either hyper- or hypo-osmotic treatment (Lew 1996). Indeed, at least in *Arabidopsis thaliana* the electrical changes coincide with changes in the net ionic fluxes that contribute to turgor recovery after hyperosmotic treatment (Shabala and Lew 2002). These electrical changes are an important part of the cell's response to osmotic stresses, but their significance is even greater, because of the implications for the use of patch clamp to measure ionic properties of plant and fungal cells.

6.1.1 Protoplasts are required for patch clamp

Patch clamp (Hamill et al. 1981) revolutionized the study of ion transport in cells, including animal, fungal, algal and higher plant cells. The two major discoverers, Erwin Neher and Bert Sakmann, were awarded the Nobel Prize in 1991. The power of the patch clamp technique was 2-fold. First, it allowed individual ion channels to be measured in situ, in their natural states in the membrane. Second, with the whole cell mode, it enabled the experimenter to extend the range of possible measurements: to examine the voltage and time dependence of ionic currents and use this information to identify the specific ions contributing to the current. With patch clamp, a wealth of information has been uncovered about the molecular foundations of ionic transport in

York University, Department of Biology, Toronto, Ontario M3J 1P3, Canada

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cells. However, when the patch clamp technique is applied to walled cells, the wall must first be removed to expose the plasma membrane to the patch pipette. To avoid lysis, the cell must be held in a solution of osmolarity high enough to induce plasmolysis. This is necessary whether the wall is removed by enzymatic digestion or some other technique, such as laser ablation. This means that the advantage of patch clamp, to examine the voltage and time dependence of ion transport across the plasma membrane, is offset by the non-physiological condition of the cell, plasmolyzed and probably attempting turgor recovery. Certainly not growing, certainly in an abnormal physiological state, the plasmolyzed state of the cell is a technical problem that obscures the relevance of patch clamp measurements. The ideal way to overcome this is to perform measurements of the voltage and time dependence of ionic currents in intact, turgid, possibly even growing cells. But how can this be done in an intact cell?

6.2 Voltage clamping intact turgid cells

Voltage clamp is the technique of choice to measure the voltage and time dependence of ionic currents across the plasma membrane. In essence, the electronics are designed to inject a current sufficient to maintain the voltage of the plasma membrane at a specified level. Data for a number of different clamped voltages are compiled to create a current versus voltage relation, or current is monitored at a single voltage over time to measure time dependence, or both may be combined. It is useful to measure the cell membrane potential concurrent with current injection into the cell to confirm the fidelity of voltage clamping. There are three ways to voltage clamp intact turgid cells: discontinuous voltage clamp, dual impalements, and double barrel micropipettes. In all instances, the intent is to measure the voltage and time dependence of the plasma membrane ionic currents separate from any contribution of the micropipette itself. The micropipette resistance is a significant problem, because the resistance at the tip of the micropipette is often similar in magnitude to the resistance of the plasma membrane. This can cause an inability to separate the voltage and time dependence of ionic currents through the micropipette tip from the ionic currents through the plasma membrane.

6.2.1 Discontinuous voltage clamp: a single barrel used for both current injection and voltage monitoring

Finkel and Redman (1984) described the discontinuous single microelectrode voltage clamp technique. The technique has been used successfully in intact higher plant cells. The basic idea is that the time dependence of electrical currents at the microelectrode tip is very different from those of the plasma membrane because the capacitance of the micropipette tip is much lower