The state of plasma mebrane polarization in plant cells

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Chapter VIII

Summary

The electrogenic characteristics of the plasma membrane of root cortex protoplasts have been investigated. Ion channel characteristics were studied, both in single patches and in whole cell configuration. The data were described with kinetic models (Chapt.V) and compared with channel and pump behavior in intact root cells (Chapt.VI). Isolated protoplasts and cells in vivo exist in two states of membrane polarization (Potassium or K-state and Pump or P-state). The state of the cell is determined by the balance between the plasma membrane H^+-ATPase and the f-K^+-channel activity (Chapt.IV)

Protoplast isolation methods for patch clamp measurements

Chapters II and III describe two different protoplasts isolation procedures, a fast procedure at 30 °C and a slow one slow at 12 °C. Both methods produced protoplasts, suitable for patch clamp experiments in the whole cell and the single patch configuration:

The first method (Chapt.II) is a convenient and rapid isolation procedure, that works especially well on roots of tomato (Lycopersicum esculentum) and Plantago species, grown on hydroculture. The procedure is based on a minimal exposure of cells to cell wall degrading enzyme mixtures. Therefore, centrifugation steps were omitted from the isolation procedure. Firstly, root material was cut and exposed to an enzyme-free solution, that contained a high concentration of mannitol, causing fast plasmolysis of the cells. Thereafter, the cells were directly placed in an enzyme mixture. After 30 minutes incubation at 30 °C all free floating cells were discarded. Subsequently, the root material was rinsed and a second group of cells, still present inside the tissue, was freed by application of mechanical pressure. The newly released protoplasts were filtered and collected on the glass bottom of a patch clamp dish.

In the second and slower isolation procedure (Chapt.III), both osmotic shock and centrifugation steps were omitted to further restrict mechanical stress. Protoplasts were isolated from different monocotyledonous and dicotyledonous plant species and different tissues (leaves and roots). The cells were incubated for a period of 12 to 14 hours at 12 °C.

Characterization of single channels

Ion channels in the plasma membrane of root cell protoplasts of Plantago media L. were studied with the patch clamp technique in the cell attached patch and outside out patch configuration (Chapt.IV). An outward rectifying potassium channel was dominantly present in the plasma membrane. It appears responsible

for the diffusional part of the cell membrane potential, dominated by the K^+-diffusion potential (E_K). This channel is activated at potentials near to and more positive than the K^+-diffusion potential. The dependence of this ion channel on K^+-activity and voltage has been characterized. The current-voltage relationships of the open channel at various K^+-concentrations are described by a four-state kinetic model. The membrane potential of intact protoplasts appears either to be dominated by the K^+-diffusion potential, the protoplast is then said to be in the K-state, or by the pump potential, generated by the plasma membrane bound proton pump / H^+-ATPase, the P-state. An experimental procedure is described to determine in cell attached patch mode the state of the protoplast, either K- or P-state.

Analysis of dynamic whole cell currents

Two different, simultaneously activated outward rectifying, K^+-currents were analyzed in the plasmalemma of root cortex protoplasts of Plantago media (Chapt.V). Their gating was E_K dependent. The threshold potential is more negative than E_K, allowing small inward currents at potentials below E_K, thereby keeping cells with little pump activity in the K-state. Time and voltage dependence of the outward rectifying K^+-currents were analyzed with Hodgkin-Huxley-like (HH) models (Hodgkin & Huxley 1952). Dynamic responses of whole cell currents to pulse potentials were analyzed with two voltage-dependent functions, the Boltzmann distribution for open probability per gate and the transition rate towards the open state (α). The transition rate in the opposite direction (ß), was calculated from α and the Boltzmann distribution. These functions were used for an integral analysis of activation and deactivation currents, measured over a range of pulse potentials. Both whole cell and single channel data were used for the determination of the number of closed and open states. The effects of single channel flickering on time response and amplitude of tail currents were added to the model. The dominant K^+-channel, present in the plasmalemma of P.media, has a characteristic non-linear single channel I/V-curve, reducing the amplitude of whole cell currents at positive potentials. To compensate for this non-linearity, a four-state translocator model had to be added to the whole cell open probability model. The analysis provides a general basis for the study and comparison of K^+-channel kinetics in plant protoplasts.

An interplay between the H^+-pump and K^+- channels

Fusicoccin (FC), an activator of the plasma membrane bound proton pump or pm-H^+-ATPase in plant cells, triggers hyperpolarization of the membrane potential (E_m), uptake of K^+ and acidification of the medium. The role of K^+-channels in activation and the effect of fusicoccin on the interaction between the potassium diffusion state (K-state) and pump state (P-state) was investigated, comparing intact root cells and isolated protoplasts of Plantago media (Chapt.VI).

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Cell attached patch clamp experiments previously showed, that isolated root cortex protoplasts are either in the K-state or in the P-state as found for intact cells. In contrast herewith, protoplasts in whole cell (WC) patch clamp experiments always were in the K-state, unless FC was added. In roots of intact plants, FC caused a decrease of the pH of the outer medium, with a concurring decrease of the K^+-concentration. The H^+ and K^+ fluxes reached a maximum, 20 to 40 minutes after FC addition. FC hyperpolarized E_m in roots, shifting the cells from K- to P-state. FC induced a similar shift in WC patch-clamp experiments, showing that FC caused an increase of the outward current by activating the pm-H^+-ATPase. The kinetics of the FC-induced processes in isolated protoplasts were the same as those in roots of intact plants. In current and voltage clamp, protoplasts in the FC-induced P-state, could be electrically shifted from P-state to K-state.

Reference: Hodgkin AL & Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. J.Physiol. 117: p500-544