Electrocoupling of Ion Transporters in Plants

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Abstract. In the plasmalemma of plants, the major ion transporters are voltage gated. Hence, they are intrinsically coupled via the membrane voltage. Theoretical predictions and electrophysiological recordings on guard cells demonstrate nonlinear oscillations of a dynamic system which provides long-term osmotic adjustment by switching between periods of net uptake and net release of salt, rather than by a steady-state.

Key words: Guard cells — Ionic relations — Nonlinear network — Membrane voltage — Oscillations — Osmosis

Introduction

In plants, osmotic and electrical relations are closely linked by the ion transporters in the plasmalemma. For the study of both subjects, guard cells play an outstanding role—first, because their physiological function consists of osmotic volume changes, and second because they are plasmatically isolated from surrounding cells, which renders them exceptionally well suited for quantitative electrophysiology (recent review: Blatt, 1991). Consequently, our knowledge about individual devices for ion transport in plants, in general, is closely related to many specific investigations which have been carried out on guard cells. Today, intact guard cells as well as their protoplasts are standard systems in plant electrophysiology. In particular, the major transporters in guard cells are well characterized with respect to the voltage dependence and relaxation kinetics of their activities, as investigated by voltage-clamp studies. However, under physiological conditions, namely at nonclamped, free-running voltages, the transporters will strongly interact with each other, because the conductance change of, let’s say, one device, will change the membrane voltage which, in turn, changes all voltage-dependent conductances, and so on. These physiological interactions and their osmotic consequences have frequently been recognized on a qualitative level. This study provides a framework of how to treat these relations quantitatively. It will start with the qualitative outline of a physiological problem and its solution. This solution will then be worked out quantitatively, and finally be confirmed by new experimental data.

There is a physiological problem in plants: the "resting voltage," $V_r$, falls into two ranges, $V_{r1}$ and $V_{r2}$, of high probability separated by a range, $V_{0r}$, of low probability. $V_{r1}$ is considerably more negative than $E_K$ (equilibrium voltage for $K^+$), and indicates the operation of an electrogenic pump with an equilibrium voltage $E_F \ll E_K$; and $V_{r2}$ is little but significantly more positive than $E_K$. Neither $V_{r1}$ nor $V_{r2}$ can reflect a long-term physiological steady-state, because there would be permanent salt uptake at $V_{r1}$ and salt loss at $V_{r2}$. However, long-term osmotic balance could be achieved by appropriate switching between $V_{r1}$ and $V_{r2}$. Voltage-mediated coupling between the transporters with their particular kinetic properties provides the physical basis for such transitions which have been observed in many plant cells, such as marine algae (Gradmann, 1976; Bisson & Kirst, 1980), glycophytic cells (Shimmen, Kikuyama & Tazawa, 1976) including guard cells (Thiel, MacRobbie & Blatt, 1992), and fungi (Slayman, Long & Gradmann, 1976).

In more detail: the following four major ion transporters of plants are known to operate in guard cells: an $H^+$ pump (Blatt, 1987), an outward-rectify-
ing K⁺ channel (Schroeder, 1988), an inward-rectifying K⁺ channel (Schroeder, 1988), and a Cl⁻ channel (Keller, Hedrich & Raschke, 1989; Hedrich, Busch & Raschke, 1990). In addition, a device for Cl⁻ uptake needs to be postulated for a physiologically complete set of transporters (MacRobbie, 1988). The most likely device is a symporter for (2H⁺-Cl⁻)⁺, which is well described for Chara (Beilby & Walker, 1981; Sanders & Hansen, 1981; Sanders, 1984). The kinetic properties of these five transporters can be summarized as follows: upon positive going voltages, the conductances of the pump, of the K⁺ importer and of the (2H-Cl⁻) symporter decrease, whereas the conductance of the K⁺ exporter increases and the conductance of the Cl⁻ channel reacts with a fast increase followed by a slow decrease. An appropriate description of the physiological situation requires an integrated model in which all relevant properties of all these transporters are considered simultaneously (Lew & Bookchin, 1986; Mummert & Gradmann, 1991). For this purpose, we use a formalism (Mummert & Gradmann, 1991) which allows us to calculate the interactions among these transporters by their coupling via the free running Vm.

Our approach to calculating the temporal behavior of nonlinear networks is a very general one. It is only worked out here numerically for the case of ion transport through plant membranes, in particular through the plasmalemma of guard cells.

Some aspects of this study are communicated in a separate context (Thiel & Gradmann, 1993).

Materials and Methods

Calculations

For each transporter, we consider one active state, A, and one inactive state I: I ↔ A; only the Cl⁻ channel has two closed inactive states, I₁ and I₂, corresponding to a fast and a slow equilibration with A: I₁ ↔ A ↔ I₂. The apparent rate constants, k, for the transitions from I to A (kA) and reverse (kᵢ) are assumed to depend on the transmembrane voltage, Vm, in the form: k = k₀exp(-δV/F/R(T)), where the superscript 0 denotes the value for k at zero voltage, and the factor 2 reflects the assumption of symmetry of the Eyring barrier. In addition, we define δ = 1, if positive-going Vm favors A by an increase of kA, and vice versa. The rate equations for the Cl⁻ channel are:

\[ \frac{dA}{dt} = -kᵢ \cdot I + k₁ \cdot A \]
\[ \frac{dI}{dt} = kᵢ \cdot A - k₁ \cdot I \]
\[ \frac{dI₁}{dt} = -kᵢ₁ \cdot I₁ + k₁₁ \cdot I₁ \]
\[ \frac{dI₂}{dt} = kᵢ₁ \cdot I₁ - k₁₁ \cdot I₂ \]

and for all other transporters

The conductance g of each transporter is the product of its maximum conductance and its probability, Pₐ, to be active, g = gₘᵢₓ \cdot Pₐ. In general, the steady-state conductances are

\[ g = gₘᵢₓ/(1 + kᵢ/k₁) \]

where gₘᵢₓ was assumed to be 1 Sm⁻¹ for all transporters, indicating that the conductances are, at least part time, of the same order of magnitude. The steady-state conductance of the Cl⁻ channel is

\[ g = 1/(1 + kᵢ/kᵢ₁ + kᵢ₂/k₁₂) \]

For the slow processes discussed here, the membrane capacitance can be ignored.

To describe the behavior of the five parallel batteries with their voltage and time-dependent conductances under free-running voltage, we start with steady-state voltage-clamp conditions (Vm₀), where the occupancy P_i of all states is stable (dP_i/dt = 0).

Upon release of voltage-clamp conditions, the voltage jumps from Vᵢ₀ to

\[ Vᵢ = (Σ(gᵢ₀ \cdot Eᵢ))/Σgᵢ₀ \]

At this voltage, all rate constants change immediately from k(Vᵢ₀) to k(Vᵢ), and all P_i relax from P_i(Vᵢ₀) towards new steady-state values P_i(Vᵢ) with the velocities dP_i/dt given by Eqs. (1). Thus, after a small time increment δt, the occupancies P_i will have changed by δP_i = P_i(Vᵢ) - P_i(Vᵢ₀) yielding Vᵢ₊₁ as calculated by Eq. (3) with new g values from g = gₘᵢₓ \cdot P_i. This procedure can now be repeated, as k(Vᵢ) will have changed to k(Vᵢ₊₁) and P_i(Vᵢ) will relax towards a new P_i(Vᵢ₊₁) by δP_i₊₁ within the next δt (Eq. (1)) resulting in a new Vᵢ₊₁, by Eqs. (2) and (3). This iterative procedure provides the time courses of the free-running V_m, plus conductances g_i (Eqs. 2), currents i = g_i(Vm - Eᵢ), fluxes Φ_i = i/F, or concentration changes ΔC_i = (a/v)ΣΩdΦ_i

dt, where a/v is the surface/volume ratio of the compartment under investigation.

Electrical Recordings

Measurements of the transmembrane voltage in guard cells of Vicia faba have been carried out as described previously (Thiel et al., 1992). The present recordings refer to a stoma width of 9 μm; the experimental chamber was rapidly perfused with experimental medium (5 mM Ca²⁺ - MES/pH 6.1 plus 5 or 15 mM KCl).

Results

A comprehensive presentation and discussion of the possible effects of the model cannot be given here. For our purpose, some selected issues are treated. The set of parameters in the Table reflects one example of realistic estimates with respect to some simplifying assumptions, i.e., identical and linear maximum conductances, first-order reactions, and