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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_{r0}, 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 $E_P \ll E_K$; 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-

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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 $(2H^+\text{-}Cl^-)^+$, 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 $(2H\text{-}Cl)^+$ 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 V_m.

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: $I \leftrightarrow A$; only the Cl^- channel has two closed inactive states, I_f and I_s, corresponding to a *f*ast and to a *s*low equilibration with A: $I_f \leftrightarrow A \leftrightarrow I_s$. The apparent rate constants, k, for the transitions from I to A (k_A) and reverse (k_I) are assumed to depend on the transmembrane voltage, V_m, in the form: $k = k^0\exp(\pm\delta V_m F/(2RT))$ for the forward (+) and reverse(−) direction, 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 $\delta = 1$, if positive-going V_m favors A by an increase of k_A, and *vice versa*. The rate equations for the Cl^- channel are:

$$dI_f/dt = -k_{Af} \cdot I_f + k_{If} \cdot A, \quad (1a)$$

$$dA/dt = k_{Af} \cdot I_f - (k_{If} + k_{Is}) \cdot A + k_{As} \cdot I_s, \quad (1b)$$

$$dI_s/dt = + k_{Is} \cdot A - k_{As} \cdot I_s \quad (1c)$$

and for all other transporters

$$dA/dt = -k_I \cdot A + k_A \cdot I \text{ and} \quad (1d)$$

$$dI/dt = k_I \cdot A - k_A \cdot I \quad (1e)$$

The conductance g of each transporter is the product of its maximum conductance and its probability, P_A, to be active, $g = g_{max} \cdot P_A$. In general, the steady-state conductances are

$$g = g_{max}/(1 + k_I/k_A) = 1/(1 + k_I/k_A) \quad (2a)$$

where g_{max} was assumed to 1 Sm^{-2} 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

$$g = 1/(1 + k_{If}/k_{Af} + k_{Is}/k_{As}). \quad (2b)$$

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 ($V_{t<0}$), where the occupancy P_i of all states is stable ($dP_{i,t<0}/dt = 0$).

Upon release of voltage-clamp conditions, the voltage jumps from $V_{t\leq 0}$ to

$$V_{t=0} = \Sigma(g_{i,t<0} \cdot E_i)/\Sigma g_{i,t<0}. \quad (3)$$

At this voltage, all rate constants change immediately from $k(V_{t<0})$ to $k(V_{t=0})$, and all P_i relax from $P_i(k_{t<0})$ towards new steady-state values $P_i(k_{t=0})$ 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 $\Delta P_{i,1}$ to $P_{i,t=1} = P_{i,t=0} + \Delta P_{i,1}$ yielding $V_{t=1}$ as calculated by Eq. (3) with new g_i values from $g = g_{max} \cdot P_A$. This procedure can now be repeated, as $k(V_{t=j})$ will have changed to $k(V_{t=j+1})$ and $P_i(k_{t=j})$ will relax towards a new $P_i(k_{t=j+1})$ by $\Delta P_{i,j+1}$ within the next Δt (Eq. 1) resulting in a new $V_{t=j+1}$ 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 $i_i = g_i(V_m - E_i)$, fluxes $\Phi_i = i_i/F$, or concentration changes $\Delta C_i = (a/v)\Sigma d\Phi_i \cdot dt$, 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 Ca^{2+} − 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

Table. Simplified kinetics of the main ion transporters in plasmalemma of guard cells[a]

Transporter		E/mV	k_A^0/sec^{-1}	k_I^0/sec^{-1}	δ	Reference
Electrogenic H^+ pump		−400	1	40	−1	Blatt, 1987
K^+ inward rectifier		−100	1	100	−1	Schroeder, 1988
K^+ outward rectifier		−100	10	1	1	Schroeder, 1988
Cl^- channel	fast	100	50	0.5	1	Keller et al., 1989;
	slow		0.1	1	−1	Hedrich et al., 1990
$(2H\text{-}Cl)^+$ symporter		200	0.015	1	−1	Beilby & Walker, 1981

[a] Data not taken from references but assumed according to them. E: Nernst equilibrium voltage; k_A^0 and k_I^0: rate constant for activation and inactivation at zero voltage; δ: Boltzmann coefficient in the expression $\exp(\delta z u)$ with z equal to the charge number (here $z = 1$) u equal to the reduced voltage, VF/RT.

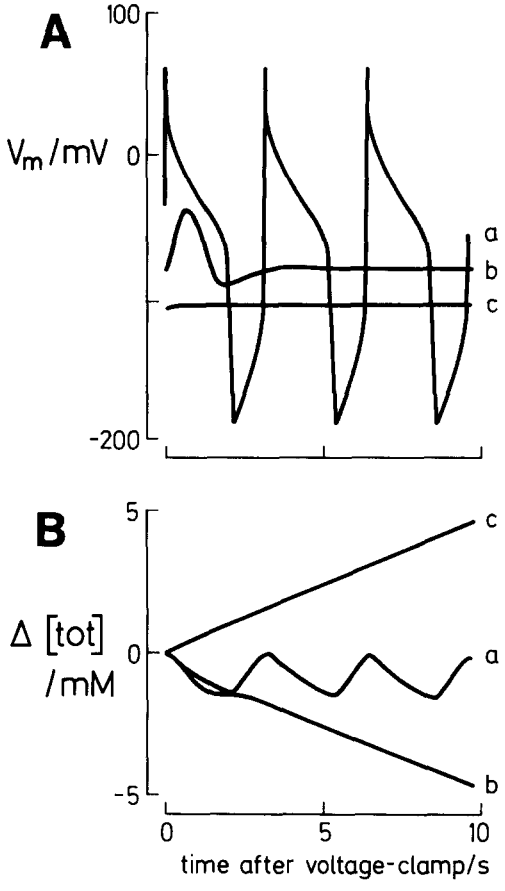

Fig. 1. Time courses of the membrane voltage (*A*) and of changes in total ion concentration (*B*), after steady-state voltage-clamp at −100 mV, calculated by algorithm in Materials and Methods, parameters as in the Table, and a surface/volume ratio of 10^{-6} m^{-1}; *a*: parameters as in the Table; *b*: same parameters as in *a*, except 40-fold values for k_A and k_I of symporter; *c*: same parameters as in *b*, except k_I^0(pump) = 5 sec^{-1}.

rounded parameter values. With these parameters, the used algorithm (*see* Materials and Methods) yields the oscillatory time courses of the membrane voltage and of the conductances of the individual elements (Fig. 1*Aa* and Fig. 2*A*) after release of voltage-clamp conditions. With these data and the knowledge of the equilibrium voltages, currents and fluxes can be calculated immediately as well as concentration changes for a given surface/volume-ratio (here 10^6 m^{-1}) which are plotted in Figs. 1*B* and 2*B*.

The system does not oscillate anymore when, in our example, the rate constants for activation and inactivation of the $(2H\text{-}Cl)^+$ symporter are increased simultaneously by a factor of forty (Figs. 1*A* and *B*, traces *b*), which is equivalent to a lowering of the activation energy between the active and the inactive state of the symporter by about 10 kJ mol^{-1}. Although this change appears to be only kinetic and not thermodynamic, the long-term effects show a significant difference, as conditions *a* yield osmotic balance, and conditions *b* osmotic loss (Fig. 1*B*). This effect corresponds in electronics to generation of DC-signals by nonlinear filtering of AC-signals.

While conditions *b* in Fig. 1 result in damped oscillations before a new steady-state is reached after voltage clamp, stimulation of the pump by reducing its inactivation by a factor of eight (conditions *c*), does not only drive the membrane voltage more negative, it suppresses the (damped) oscillations almost completely. The traces with nonoscillatory characteristics (*b* and *c* in Figs. 1*A* and *B*) show that only minor deviations of the steady-state voltage from E_K results in considerable net uptake or loss of salt.

Details of the oscillating system (traces *c* in Figs. 1*A* and *B*) are plotted in Fig. 2, including the behavior of the electrical parameters (*A*) and of the osmotic consequences (*B*). The changes in cellular $[H^+]$ as calculated for an unbuffered system ($\Delta[H^+]$ in Fig. 2*B*) are expected to be essentially neutralized by the buffering system and the acid metabolism of the cell.

Figure 3 shows a comparison between the model oscillations (*A*) and the voltage oscillations (*B*) measured in guard cells of *Vicia faba*. To match the time scales, all rate constants are half of those in the Table (except k_I of the pump which was set to 10 sec^{-1} to stabilize the oscillations), and the first peak depolarization was drawn to be in phase in the upper and in the lower panel.

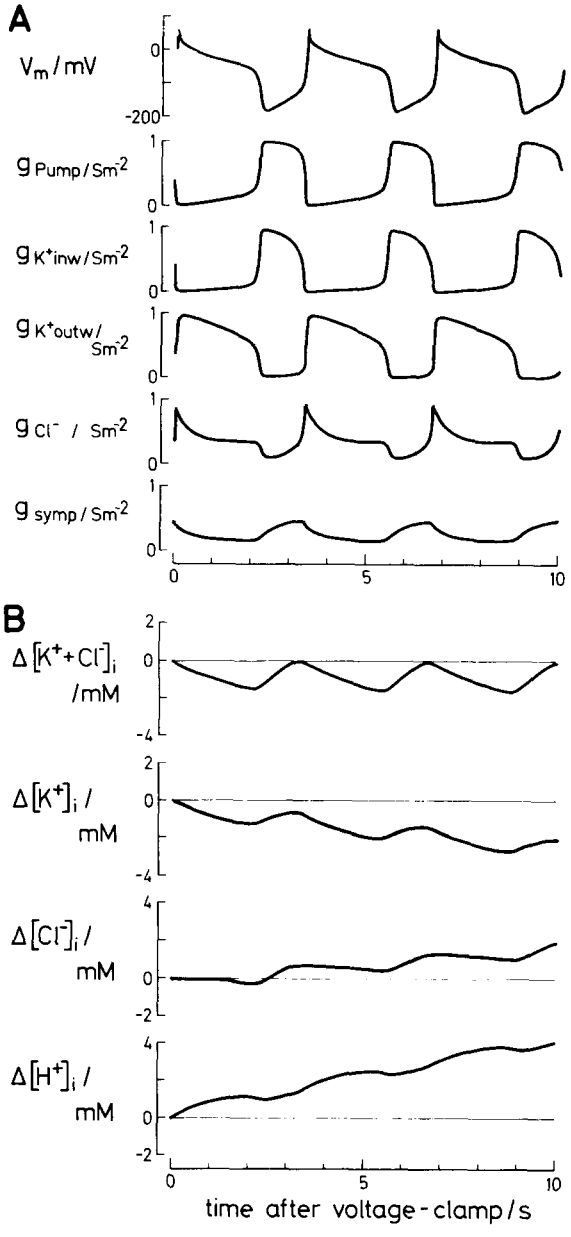

Fig. 2. Dynamic behavior of individual transport functions during nonlinear oscillations; details to Fig. 1*a*; (*A*) electrical parameters: membrane voltage and conductances; (*B*) osmotic parameters: global and individual concentration changes; given changes in $[H^+]_i$ for unrealistic, unbuffered system; realistic assumption: $[H^+]_i$ osmotically irrelevant.

There are obvious similarities between the shapes of the nonlinear V_m oscillations of the model and of the experimental ones: the positive maxima are sharper than the negative ones, the depolarization is regenerative with one inclination point, and the (re)polarization is sigmoidal with two inclination points, showing a shoulder (plateau) 10 mV more positive than E_K ($-60\,mV \geq E_K \geq -100\,mV$). Model and measurements confirm the statement in the Introduction about two voltage ranges of high probability, one slightly more positive than E_K, and one much more negative.

Furthermore, the effect of external [KCl] has been investigated both on the physiological response and on the model characteristics (upper and lower panel in Fig. 3). A threefold increase of external [KCl] has been accounted for in the model by corresponding 30 mV changes of the equilibrium voltages of the two K^+ channels, of the Cl^- channel and of the $(2H\text{-}Cl)^+$ symporter, plus by a global conductance increase of 50% for these transporters. Both the experimental and the model oscillations turned out to slow down by about 15% upon a threefold increase in external [KCl].

Discussion

Specific aspects

We have pointed out in the Introduction that steady-state voltages, slightly more negative than E_K (V_{r0}), are seldom; thus, the results in Fig. 1 from conditions *c* may be considered untypical. On the other hand, this example demonstrates well that even a small, steady-state driving force in the form of a membrane voltage of only about 7 mV more negative than E_K, can create a considerable concentration increase, here by 1.8 M per hour. Even if our assumptions were an order of magnitude off, e.g., by overestimates of the maximum conductances and/or of the surface/volume ratio, permanent osmotic changes of around 200 mM per hour were intolerable for guard cells, not to speak of larger driving forces. The same problem arises if measured steady-state fluxes of some 100 $nmol \cdot m^{-2} \cdot sec^{-1}$ (Thiel et al., 1992) are considered.

In our example (Fig. 2), the conductance of the $(2H\text{-}Cl)^+$ symporter does not exceed 0.5 Sm^{-2}, in contrast to the other devices. However, the currents and fluxes (*not illustrated*) are in the same range because of the large driving force of the symporter. There is an acute challenge to identify the Cl^- uptake system in guard cells, since the Cl^- releasing channels are known in great detail (Keller et al., 1988; Hedrich et al., 1990; Mummert & Gradmann, 1991).

General aspects

The time courses of the electrical parameters (Fig. 2*A*) illustrate some relationships of the system which could also be demonstrated by steady-state considerations.

As for ion uptake, three reactions are involved: ATP-driven H^+ export provides the driving force

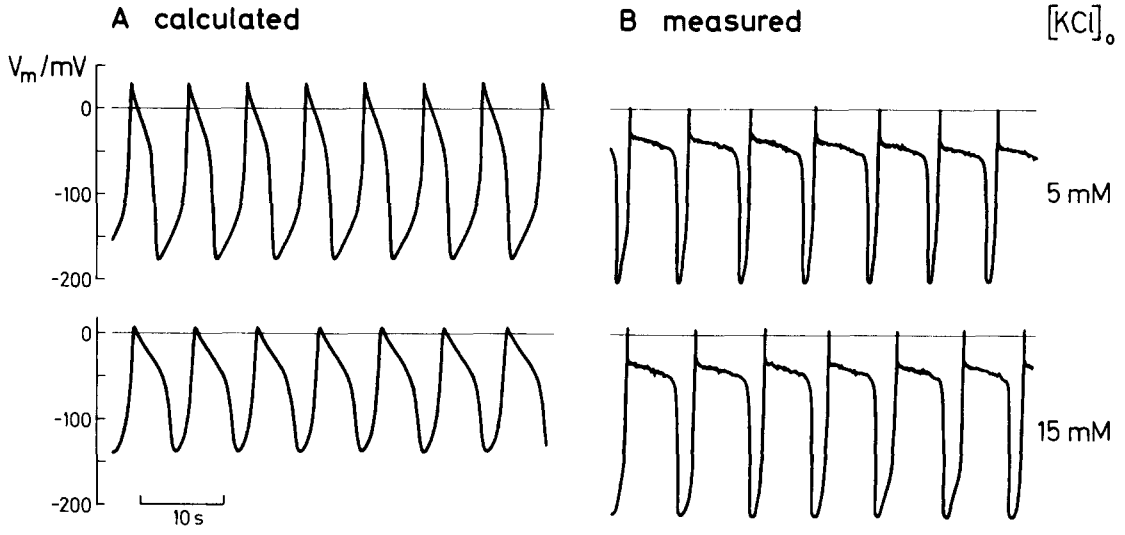

Fig. 3. (*A*) Spontaneous, nonlinear oscillations of membrane voltage in low (upper panel) and high (lower panel) external KCl. (*A*) model calculations, low KCl conditions with all k values half of that in the Table, and k_f(pump) = 10 sec^{-1}; model parameters for threefold external [KCl]: same as control, except E_K = −70 mV, E_{Cl} = 70 mV and $E_{Symporter}$ = 170 mV, and g_{max} of symporter, K^+ and Cl^- channels increased from 1 to 1.5 Sm^{-2}. (*B*) Two exemplary sections from a continuous, 13.6 min, recording of oscillating membrane voltage in one intact *Vicia faba* guard cell.

for the import of $(2H\text{-}Cl)_o^+$ and of K^+, resulting in a $[H^+]$ and electroneutral overall uptake of KCl driven by ATP, if the stoichiometric relations between these processes were: 2ATP/2H : $(2H\text{-}Cl)^+$: K^+. Figure 2*B* (especially the H^+ tracing) shows, however, that at realistic, noninteger relations between the transport processes, the overall process will not be pH neutral any more. In realistic terms, however, these pH changes are expected to be essentially neutralized by the buffering system and the acid metabolism of the cell. The latter can be expected to have additional osmotic effects which are ignored at the moment.

Loss of salt requires voltages more positive than E_K which can only occur with some activity of one or several devices with an equilibrium voltage more positive than E_K, namely the $(2H\text{-}Cl)^+$ symporter and/or the Cl^- channel in our model.

The problem of osmotic steady-state balance at a resting voltage and its solution by appropriate switching of a bistable system, between uptake at high voltage (V_{r1}) and release at low voltage (V_{r2}), seems to be a fundamental mechanism in plant cells. It may be understood as the origin of electrical excitation in eukaryotes, which has been suggested before to be more a matter of osmoregulation (Mummert & Gradmann 1976; Gradmann & Mummert, 1980) than of long distance transmission of information as in excitations of nerves and muscles. Notably, animal action potentials are, in contrast to plant cells, osmotically neutral. During an action potential in plant cells, Cl^- and K^+ are released from the cell. Since in plant action potentials the ion fluxes are much larger than required for the electrical phenomenon (Mummert & Gradmann, 1991), action potential in plants could well be an epiphenomenon of an osmotic event, whereas in animals the electrical phenomenon has gained priority over the osmotic ones.

Compared to our view about the general impact of voltage oscillations in plants, there are few reports about spontaneous transitions between V_{r1} and V_{r2} and *vice versa*. One possibility to account for this discrepancy is, that many experimenters (like us), who observe such transients, have chosen their experimental conditions to avoid these phenomena which counteract the desire for reproducible and comprehensible steady-state results.

Unfortunately, the classical approach of describing dynamic electrical membrane phenomena in terms of a parallel arrangement of batteries with voltage- and time-dependent conductances, has so far been applied only a few times (Beilby & Coster, 1979; Mummert & Gradmann, 1991) by plant electrophysiologists. With the increasing availability of electrical data on ion transporters and of computer-aided calculations, this approach is expected to gain importance.

In principle, our model could be extended by known specifications of the transporters, by additional transporters, e.g., the 'slow' Cl^- channel (Linder & Raschke 1992; Schroeder and Keller, 1992), and by processes and factors (e.g., cytoplasmic Ca^{2+}), which control the activity of the

transporters. However, we consider such extensions not useful at the moment, because the model in its present form is already too complex to yield reliable predictions with respect to alternate sets of reasonable system parameters. Rather, the approach justifies a general idea in quantitative terms.

Conclusions

Based on literature data, on our calculations, and on our measurements, we conclude that long-term osmotic balance in plants is accomplished, not by a steady-state but by transitions between two stable states, one of salt uptake at voltages considerably more negative than E_K, and another one of salt release at voltages some 10 mV more positive than E_K.

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