

A Mathematical Model of Action Potential in Cells of Vascular Plants

Vladimir Sukhov · Vladimir Vodeneev

Received: 20 June 2009 / Accepted: 23 October 2009 / Published online: 17 November 2009
© Springer Science+Business Media, LLC 2009

Abstract A mathematical model of action potential (AP) in vascular plants cells has been worked out. The model takes into account actions of plasmalemma ion transport systems (K^+ , Cl^- and Ca^{2+} channels; H^+ - and Ca^{2+} -ATPases; $2H^+/Cl^-$ symporter; and H^+/K^+ antiporter), changes of ion concentrations in the cell and in the extracellular space, cytoplasmic and apoplasmic buffer capacities and the temperature dependence of active transport systems. The model of AP simulates a stationary level of the membrane potential and ion concentrations, generation of AP induced by electrical stimulation and gradual cooling and the impact of external Ca^{2+} for AP development. The model supports a hypothesis about participation of H^+ -ATPase in AP generation.

Keywords Action potential · Vascular plant · Ion transport · Mathematical model

Introduction

In natural conditions plants are exposed to various environmental factors and respond to their actions by the development of a number of functional changes. Among them action potential (AP) generation and propagation are considered to be the earliest responses. At present, many researchers focus their efforts on understanding the functional role of AP in vascular plants (Fromm and Lautner 2007; Pyatygin et al. 2008). For instance, the generation

and propagation of APs are known to induce a number of changes in such physiological processes as photosynthesis, respiration, phloem transport, gene expression and others (Fromm and Lautner 2007). However, the way electrical signals are transformed into functional responses is still unclear, mainly due to a lack of understanding of the AP generation mechanism.

According to the current concept, the generation of AP is associated with passive fluxes of Ca^{2+} , Cl^- and K^+ (Samejima and Sibaoka 1982; Opritov and Retivin 1982; Fromm and Spanswick 1993; Fromm and Bauer 1994; Felle and Zimmermann 2007). This hypothesis assumes the following sequence of events during the generation of AP in vascular plant cells (Davies 2006; Trebacz et al. 2006; Felle and Zimmermann 2007): depolarization to the threshold level of the membrane potential (E_m) → activation of potential-dependent Ca^{2+} channels → influx of calcium ions into the cell → activation of potential-dependent Cl^- channels → efflux of Cl^- from the cell and development of the AP depolarization phase → activation of potential-dependent outward K^+ channels → efflux of K^+ and development of the repolarization phase. However, there are a number of facts which show participation of H^+ -ATPase in vascular plant AP generation: suppression of AP generation by inhibitors of metabolism and H^+ -ATPase (Hodick and Sievers 1988; Fromm and Spanswick 1993; Vodeneev et al. 2006), generation of AP under conditions with high concentration of external K^+ and Cl^- (Pyatygin et al. 1999a), a transitory change of extracellular pH during AP generation and a connection of this change with H^+ -ATPase regulation by calcium (Vodeneev et al. 2006), etc. There are several works (Gradmann 1976; Mummert and Gradmann 1991) which show that an electrogenic pump (Cl^- pump) takes part in AP generation in the alga *Acetabularia*. On the basis of this idea and the

V. Sukhov (✉) · V. Vodeneev
Department of Biophysics, N.I. Lobachevsky State University
of Nizhny Novgorod, Gagarin Avenue, 23, Nizhny Novgorod
603950, Russia
e-mail: vssuh@mail.ru

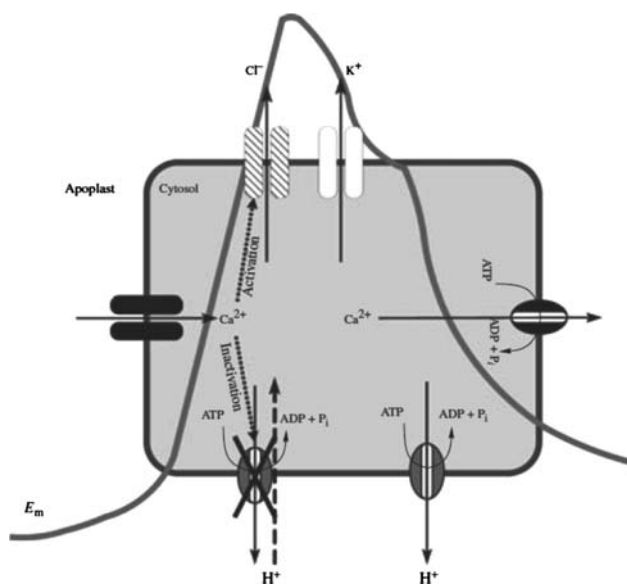


Fig. 1 Proposed scheme of AP generation in excitable cells of vascular plants that takes into account passive ion fluxes and transient changes in the activity of electrogenic H^+ -ATPase (Vodeneev et al. 2006)

experimental data on vascular plants, a modified hypothesis on the vascular plant AP mechanism has been proposed (Vodeneev et al. 2006). It assumes (Fig. 1) that the influx of calcium ions into the cell not only induces the activation of Cl^- channels but reversibly inactivates H^+ -ATPase. As a result, this inactivation can take part in AP generation.

The AP mathematical model, which takes into account Ca^{2+} , Cl^- , K^+ and H^+ transporters, can be used for a theoretical test of the hypothesis on the participation of H^+ -ATPase in AP generation. There are only few mathematical models of algal AP which include external stimuli. One of them takes into account passive fluxes and is based on the Hodgkin–Huxley model (Beilby 1982, 2007). It simulates AP in the alga *Chara*; however, possible participation of H^+ -ATPase and considerable changes of ion concentrations during AP generation in the vascular plants (Opritov and Retivin 1982; Fromm and Bauer 1994; Vodeneev et al. 2006; Felle and Zimmermann 2007) restrict the application of this model. Another model (Mummert and Gradmann 1991) takes into account the active flux (Cl^- pump) as well as passive fluxes and simulates AP generation in *Acetabularia acetabulum*. This model more accurately simulates AP generation in vascular plants; however, application of the model requires a description of ion transport systems in the plasmalemma of vascular plant cells.

On the basis of the plasmalemma electrical process model by Gradmann et al. (Gradmann et al. 1993; Gradmann and Hoffstadt 1998; Gradmann 2001a, b), a preliminary model of AP generation taking into account active and passive ion transport systems has been worked out

(Sukhov and Vodeneev 2005). The aim of this work was to improve the preliminary model and to check the hypothetical foundations (including the theoretical analysis of H^+ -ATPase participation in AP generation).

Theory

Figure 2 shows the scheme of the vascular plant cell electrophysiological model (Sukhov and Vodeneev 2005) which includes plasmalemma basic transport systems (H^+ , Ca^{2+} , Cl^- and K^+). Five of these carriers (outward and inward K^+ channels, Cl^- channels, H^+ -ATPase, $2H^+/Cl^-$ symporter) as well as cytoplasmic and apoplastic buffers have been taken into account on the basis of the model by Gradmann et al. (Gradmann et al. 1993; Gradmann and Hoffstadt 1998; Gradmann 2001a, b). The H^+/K^+ antiporter (Rodríguez-Navarro 2000) has been included in the model since it maintains a stationary concentration of K^+ at rest, when E_m is more negative than the potassium equilibrium potential (Opritov et al. 1991). Other additional elements in our model are systems of Ca^{2+} transport: Ca^{2+} channels (Zimmermann and Sentenac 1999) and Ca^{2+} -ATPase (Bush 1993) since the influx of calcium triggers the initiation of AP in vascular plant cells (Davies 2006; Trebacz et al. 2006; Vodeneev et al. 2006; Felle and Zimmermann 2007), inducing the activation of Cl^- channels and, possibly, the inactivation of H^+ -ATPase.

The changes of the membrane potential are described by the Hodgkin–Huxley equation, which has been modified for ion fluxes in plants (Gradmann et al. 1993; Gradmann and Hoffstadt 1998; Gradmann 2001a, b; Sukhov and Vodeneev 2005):

$$\frac{dE_m}{dt} = \frac{1}{C} \cdot Fj_r \quad r \in (K^+, Cl^-, H^+, Ca^{2+}) \quad (1)$$

where E_m is the membrane potential, j_r is the transmembrane ion flux r , C is the specific capacitance and F is the Faraday constant.

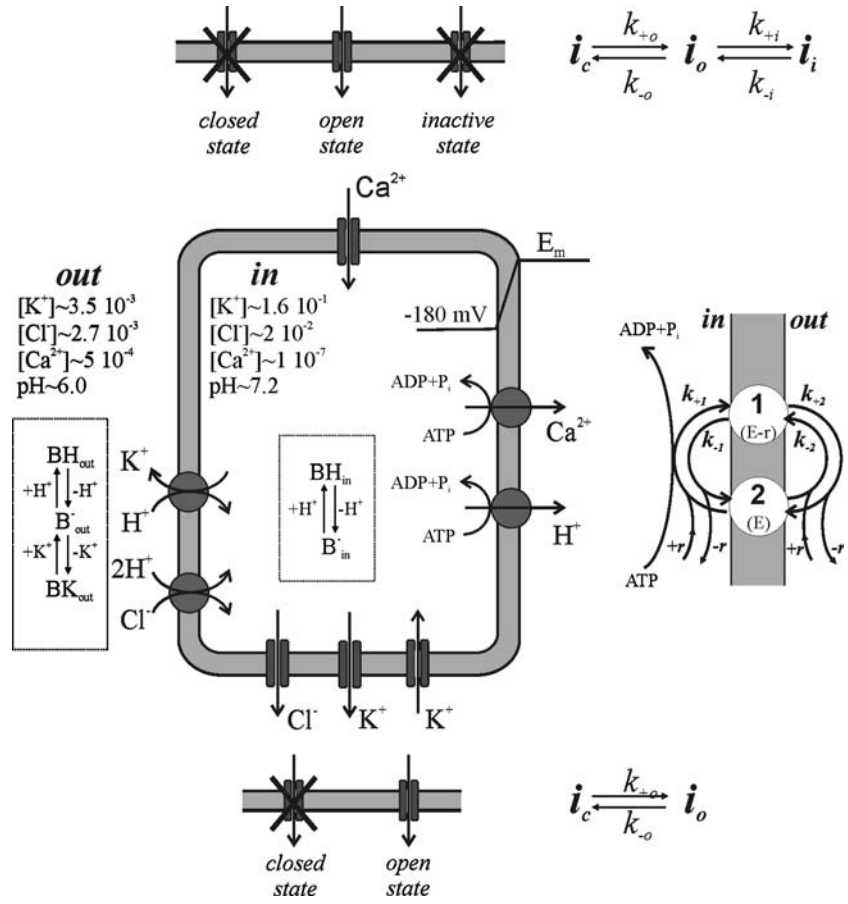
Passive ion transport is described on the basis of the Goldman–Hodgkin–Katz equation (Gradmann and Hoffstadt 1998):

$$j = p_o^r P_{\max}^r z u \frac{[r]_{in} - [r]_{out} \exp(-zu)}{1 - \exp(-zu)} \quad (2)$$

where p_o^r is the probability of the r -ion channel open state; P_{\max}^r is the r -ion maximum permeability; $[r]_{in}$ and $[r]_{out}$ are free r concentrations in the cytoplasm and apoplast, respectively; $u = FE_m/(RT)$ is the normalized membrane potential; z is the ion charge; and R and T have their standard thermodynamic values.

There are two states of K^+ and Cl^- channels in our model: the closed state and the open state (Barbier-Brygou

Fig. 2 Scheme of a plant cell electrophysiological model. E_m is the electrical potential of the plasma membrane; $[K^+]$, $[Cl^-]$ and $[Ca^{2+}]$ are the concentrations of potassium, chlorine and calcium ions, respectively (M); B_{in}^- and BH_{in} are free and H^+ -bound proton-buffer molecules in the cell; B_{out}^- , BH_{out} and BK_{out} are free, H^+ - and K^+ -bound buffer molecules in the apoplast. Above is the schema of Ca^{2+} channel states; below is the schema of Cl^- and K^+ channel states; on the right is the schema of the “two-state” model of primary active transport (see text)



et al. 2000; Gradmann 2001a, b). Ca^{2+} channels have three states: closed, open and inactivated (Huang et al. 1994). The dynamics of open state probabilities (p_o^r) are described (Gradmann and Hoffstadt 1998) by Eq. 3 for K^+ and Cl^- channels and by Eq. 4 for Ca^{2+} channels:

$$\frac{dp_o^r}{dt} = k_{+o}^r(1 - p_o^r) - k_{-o}^r p_o^r \tag{3}$$

$$\frac{dp_o^r}{dt} = k_{+o}^r(1 - p_o^r - p_i^r) - k_{-o}^r p_o^r + k_{-i}^r p_i^r - k_{+i}^r p_o^r \tag{4}$$

$$\frac{dp_i^r}{dt} = k_{+i}^r p_o^r - k_{-i}^r p_i^r$$

where p_i^r is the probability of the r -ion channel inactivated state; $k_{+o(+i)}^r = k_{o(i)}^r \exp\left(\frac{uc_{o(i)}^r}{2}\right)$ and $k_{-o(-i)}^r = k_{o(i)}^r \exp\left(\frac{c_{o(i)}^r u_{o(i)}^r - uc_{o(i)}^r}{2}\right)$ are the velocity constants of transitions from the closed state to the open one and vice versa (subscript o) or from the open state to the inactivated one and vice versa (subscript i); c_o^r and c_i^r are the constants which represent a portion of the membrane potential acting on the gating mechanisms and their charge; u_o^r and u_i^r are the normalized potential barriers for the transition of the channel from the closed state to the open one and from the open state to the inactivated one, respectively; k_o^r and k_i^r are

the constants representing transition velocities between the closed and open states and between the open and inactivated states at u, u_o^r and u_i^r equal to 0.

Equations 3 and 4 have numerically been solved for outward K^+ channels and Ca^{2+} channels. Open probabilities of Cl^- channels and inward K^+ channels are described by equilibrium distributions since activation and deactivation velocities of these channels are faster than those of membrane potential changes during AP in vascular plants (Blatt 1992; Thomine et al. 1995). The model takes into account the dependence of Cl^- channel activity on the Ca^{2+} concentration in the cytoplasm (Lewis et al. 1997; Berestovsky and Kataev 2005). The Ca^{2+} influence on Cl^- channel activity has been described by addition of coefficient A into Eq. 2:

$$A = \frac{[Ca^{2+}]_{in}^2}{K^2 + [Ca^{2+}]_{in}^2} \tag{5}$$

where A is the portion of Cl^- channels activated by Ca^{2+} , $[Ca^{2+}]_{in}$ is the Ca^{2+} concentration in the cytoplasm, K is the constant equal to $[Ca^{2+}]_{in}$ at $A = 0.5$ and the power 2 reflects activation of the Cl^- channel by two Ca^{2+} (Berestovsky and Kataev 2005). The open probability of Cl^- channels has been assumed to be very small ($P_{Ca}^o \approx 0$) for low Ca^{2+} concentrations ($< 10^{-7}$ M) (Barbier-Brygoo et al. 2000).

Primary active transport of ions (H^+ -ATPase and Ca^{2+} -ATPase) is described by the “two-state model” (Hansen et al. 1981; Beilby and Shepherd 2001; Sukhov and Vodeneev 2005), which takes into account two states of pump: with an ion and without it (Fig. 2). Unlike some other models (the ohmic description, the description on the basis of the Goldman–Hodgkin–Katz equation [Gradmann et al. 1993; Gradmann and Hoffstadt 1998; Gradmann 2001a, b]), the model not only simulates the dependence of the transport on the ion concentration and E_m but also describes the enzyme saturation by high concentrations of H^+ and Ca^{2+} as well as by the membrane potential. This can be important for the description of AP in vascular plants since substantial changes of ion concentrations during AP have been observed (Opritov and Retivin 1982; Fromm and Bauer 1994; Felle and Zimmermann 2007).

Equation 6 describes stationary active fluxes of H^+ and Ca^{2+} :

$$j_P = E_0 \frac{k_{+1}k_{+2} - k_{-1}k_{-2}}{k_{+1} + k_{-1} + k_{+2} + k_{-2}} \quad (6)$$

where E_0 is the total enzyme concentration and k_{+1} , k_{-1} , k_{+2} and k_{-2} are velocity constants of forward (+) and reverse (–) transitions between two states of the pump (indices 1 and 2 are used for potential independent and dependent constants, respectively). The Goldman–Hodgkin–Katz equation is used to describe the dependence of constants k_{+2} and k_{-2} on the membrane potential.

Constants (k) for H^+ -ATPase have been calculated using Eq. 7:

$$k_{+1} = k_1 [H^+]_{in}, \quad k_{-1} = k_1 \exp(-\Delta G_{ATP}/RT), \\ k_{+2} = k_2 u \frac{1}{1 - \exp(-u)}, \quad k_{-2} = k_2 [H^+]_{out} u \frac{\exp(-u)}{1 - \exp(-u)} \quad (7)$$

where k_1 and k_2 are the velocity constants at $u = 0$ and $[H^+]_{in} = [H^+]_{out} = 1$ M, $[H^+]_{in}$ and $[H^+]_{out}$ are the H^+ concentrations in the cytoplasm and apoplast, respectively; ΔG_{ATP} is the energy of ATP hydrolysis.

The dependence of pump activity on the Ca^{2+} concentration in the cytoplasm (Kinoshita et al. 1995; Vodeneev et al. 2006) has been taken into account in the description of H^+ -ATPase using Eq. 8:

$$A = \frac{K^n}{K^n + [Ca^{2+}]_{in}^n} \quad (8)$$

where A is the portion of H^+ -ATPase inactivated by Ca^{2+} , K is the constant equal to $[Ca^{2+}]_{in}$ at $A = 0.5$ and n is the number of Ca^{2+} needed to inactivate one molecule of the enzyme. It is assumed that $n = 2$.

Ca^{2+} -ATPase is described as a system which exchanges Ca^{2+} and H^+ (Bush 1993). Constants (k) are given by Eq. 9:

$$k_{+1} = k_1 [Ca^{2+}]_{in} [H^+]_{out}, \quad k_{-1} = k_1 \exp(-\Delta G_{ATP}/RT), \\ k_{+2} = k_2 u \frac{1}{1 - \exp(-u)}, \quad k_{-2} = k_2 [Ca^{2+}]_{out} [H^+]_{in} u \frac{\exp(-u)}{1 - \exp(-u)} \quad (9)$$

where $[Ca^{2+}]_{in}$ and $[Ca^{2+}]_{out}$ are the Ca^{2+} concentrations in the cytoplasm and apoplast, respectively.

Ion fluxes associated with secondary active transport are described as a difference between fluxes directed to the apoplast (j_{in}) and to the cytoplasm (j_{out}): $j_P = j_{in} - j_{out}$, flux velocities being proportional to ion concentrations (Eqs. 10 and 11). The electrogenic active flux of Cl^- also depends on the membrane potential (Eq. 10) (Gradmann 2001a).

$$j_P^{Cl} = V^{Cl} \left([Cl^-]_{in} [H^+]_{in}^2 u \frac{1}{1 - \exp(-u)} - [Cl^-]_{out} [H^+]_{out}^2 u \frac{\exp(-u)}{1 - \exp(-u)} \right) \quad (10)$$

$$j_P^K = V^K ([K^+]_{in} [H^+]_{out} - [K^+]_{out} [H^+]_{in}) \quad (11)$$

where j_P^{Cl} and j_P^K are the fluxes of Cl^- and K^+ and V^{Cl} and V^K are the total velocity constants of $2H^+/Cl^-$ symporter and H^+/K^+ antiporter, respectively. For ion r , $V^r = k^r E^r$, where E^r is the concentration of the enzyme which transports ion r , k^r is the velocity constant at $u = 0$ and $[r]_{in} = [r]_{out} = 1$ M.

This description does not take into account different states of transporters, so it cannot describe a number of transport system properties, such as ion transport saturation; however, it is simpler than that of the two-state model and requires only one parameter.

The temperature influence on primary and secondary systems of active transport is described by temperature coefficient Q_{10} , which has been introduced into Eqs. 6, 10 and 11 (Sukhov and Vodeneev 2005). It has been assumed that $Q_{10} = 3$, which is typical for enzymatic processes. The dependence of passive transport systems on temperature is weak; therefore, the temperature coefficient has not been used for their description.

Ion concentration changes and the buffer capacity of cytoplasm and apoplast. Changes of ion concentrations in the cytoplasm and apoplast are described on the basis of Eq. 2 by adding coefficients S_{cell}/V_{cell} (a ratio between the cell surface and the cell volume) and V_{cell}/V_{ap} (a ratio between the apoplast and cell volume) (Sukhov and Vodeneev 2005).

Gradmann’s model (2001a, b) (without the apoplast Ca^{2+} buffer since the calcium extracellular concentration

Table 1 Values of parameters and initiation concentrations of ions in the mathematical model of AP

Parameter	Value	Source	Parameter	Value	Source
General parameters			Inward K^+ channel		
C	10^{-6} F cm^{-2}	Opritov et al. 1991	P_{max}	2.90×10^{-6} cm s^{-1}	Tyerman et al. 2001
S_{cell}/V_{cell}	10^4 cm^{-1}	Opritov et al. 1991	c^o	1.10	Blatt 1992 ^a
V_{cell}/V_{ap}	0.42	Opritov et al. 1991	u^o	-7.40	Blatt 1992 ^a
B_{in}	0.200 M	Gradmann 2001a	H ⁺ -ATPase		
B_{out}	0.083	Opritov et al. 1991; Gradmann 2001a ^a	E_0	0.1M	Assumed
K_H^{in}	10^{-6} M	Gradmann 2001a	k_1	0.45 s^{-1}	Assumed
K_H^{out}	10^{-6} M	Gradmann 2001a	k_2	2.58×10^{-4} s^{-1}	Assumed
K_K^{out}	10^{-4} M	Gradmann 2001a	ΔG_{ATP}	-50 kJ	Gradmann and Hoffstadt 1998
Ca ²⁺ channel			K	4×10^{-7} M	Kinoshita et al. 1995
P_{max}	1.5×10^{-7} cm s^{-1}	Opritov et al. 1991 ^a	Ca ²⁺ -ATPase		
c_o	1.15	White 1998 ^a	E_0	0.63 M	Assumed
u_o	-3.51	White 1998 ^a	k_1	45 s^{-1}	Assumed
k_o	353 s^{-1}	Klusener and Weiler 1999	k_2	2.58×10^{-5} s^{-1}	Assumed
c_i	1.28	Assumed	K ⁺ /H ⁺ antiporter		
u_i	-7.01	Assumed	V_0^K	0.015 m ⁻¹ s^{-1}	Assumed
k_i	0.6 s^{-1}	Assumed	2H ⁺ /Cl ⁻ symporter		
Cl ⁻ channel			V_0^{Cl}	2×10^4 M ⁻² s^{-1}	Assumed
P_{max}	2.45×10^{-6} cm s^{-1}	Opritov et al. 1991 ^a	Initiation concentrations of ions		
c^o	2.57	Thomine et al. 1995 ^a	$[H^+]_{in}/[H^+]_{in}^{total}$	$7 \times 10^{-8}/0.015$ M	Opritov et al. 1991 ^a
u^o	-4.68	Thomine et al. 1995 ^a	$[H^+]_{out}/[H^+]_{out}^{total}$	$10^{-6}/0.002$ M	Gradmann and Hoffstadt 1998 ^a
K	8×10^{-6} M	Lewis et al. 1997 ^a	$[K^+]_{in}$	0.16 M	Opritov et al. 1991
Outward K ⁺ channel			$[K^+]_{out}/[K^+]_{out}^{total}$	0.0035/0.082 M	Opritov et al. 1991 ^a
P_{max}	2.9×10^{-6} cm s^{-1}	Opritov et al. 1991 ^a	$[Cl^-]_{in}$	0.02 M	Opritov et al. 1991
c^o	1.13	Pei et al. 1998 ^a	$[Cl^-]_{out}$	0.0027 M	Opritov et al. 1991
u^o	-2.53	Bentrup 1990 ^a	$[Ca^{2+}]_{in}$	10^{-7} M	Bush 1993
k_o	0.5 s^{-1}	Moran et al. 1988	$[Ca^{2+}]_{out}$	5×10^{-4} M	Opritov et al. 1991

^a Value of parameter calculated based on this work

has been assumed to be constant) has been used to simulate the buffer capacity of the cytoplasm and apoplast (the scheme of buffer states is shown in Fig. 2). The values of concentrations of H⁺ and K⁺ buffers ($[B]_{in}$ and $[B]_{out}$ for the cytoplasm and apoplast, respectively) and dissociation constants (K_H^{in} , K_H^{out} , K_K^{out} for H⁺ in the cytoplasm and apoplast and for K⁺ in the apoplast) have been taken from Gradmann (2001a).

The model parameters have been taken from the literature or assumed (Table 1; Fig. 2). In the latter case, we have used the parameter values which are typical for the transport processes in the plant cell and describe the basic properties of these processes. A quantitative agreement between model and experimental results was the second criterion for the correctness of the accepted values.

The total system of equations (on the basis of Eqs. 1–11) has been solved by Euler's method. Δt has been taken to equal 5 ms since $\Delta t < 10$ ms does not influence the

simulated results. An initial value of the membrane potential has been accepted as -180 mV (Opritov et al. 1991); initial distributions of closed, open and inactivated states have been calculated as the equilibrium ones for this value of the membrane potential; and the ion initial concentrations have been taken from the literature (Table 1).

Results and Discussion

The numerical solution of the model equations has shown that E_m and ion concentrations in the cell and the apoplast are stationary without stimulation and correspond to the real values in vascular plants (values shown in Fig. 2).

Simulated changes of E_m , induced by electrical stimulation (temporary increase of the electrical potential on the plasma membrane to various constant values), are shown in Fig. 3. The imitation of the electrical stimulation more than

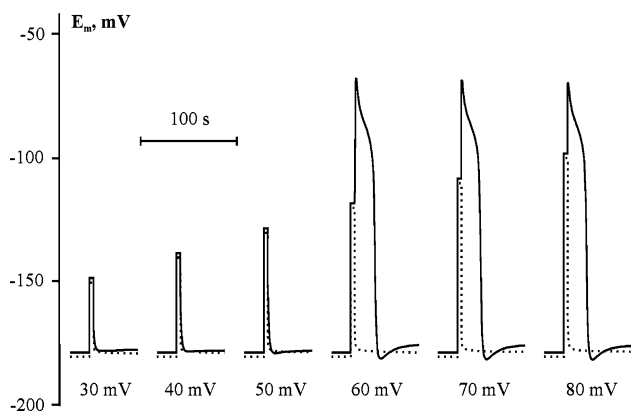


Fig. 3 APs induced by electrical stimulation with Ca^{2+} in the apoplast (5×10^{-4} M) (solid line) and without it (dashed line). Electrical stimulation was simulated by an increase of E_m to the constant level (the value of E_m changes is shown under the curve; the duration of stimuli was 4 s)

a threshold potential induces AP generation, the amplitude of which does not depend on the stimulation magnitude (Fig. 3). This result is in good agreement with the “all-or-none” law, which is an important characteristics of AP (Davies 2006; Trebacz et al. 2006). It is necessary to note that the magnitude of the threshold electrical stimulus depends on its duration: the long-term stimulus magnitude has been shown to be less than that of the short-term one. This result fits well with the classic relationship strength–duration, which is typical for excitable structures including vascular plant cells (Zawadzki et al. 1991; Fromm and Spanswick 1993).

At the next stage of the research, we investigated the influence of gradual cooling on E_m . It should be noted that gradual cooling is one of the effective methods of AP induction, which allows one to distinguish the subthreshold changes of E_m and AP generation (Pyatygin et al. 1992). Application of this method to simulate AP generation makes it possible to compare theoretical results with our

experimental data (Opritov et al. 1991, 2002; Pyatygin et al. 1999a, b; Vodeneev et al. 2006).

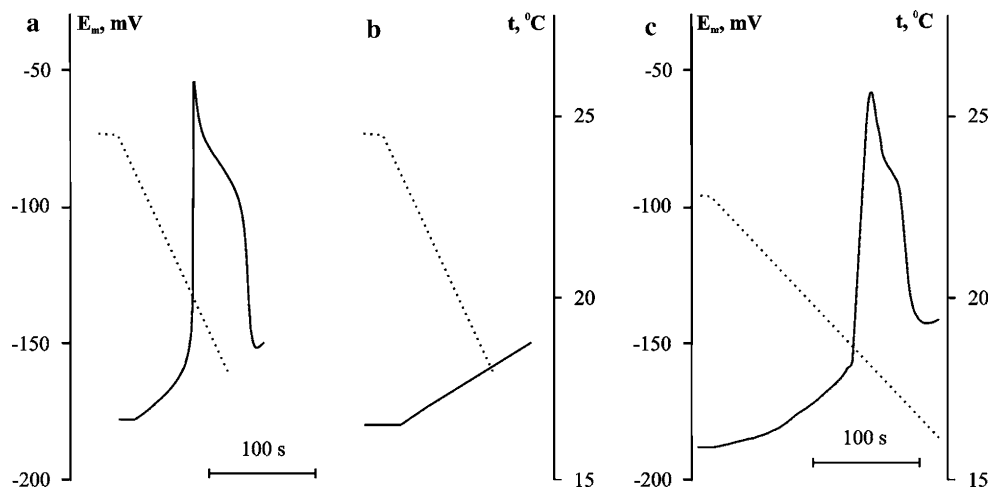
Gradual cooling is considered a linear decrease of temperature (4°C min^{-1}) from an initial value (25°C). The imitation of cooling induces typical changes of E_m : a weak depolarization up to threshold level of E_m , followed by AP generation (Fig. 4a). Simulated changes of the membrane potential are very similar to those obtained in the gradual cooling experiments (Fig. 4c).

As seen from Figs. 3 and 4a, the simulated AP has two stages of repolarization, which is typical for vascular plants (Samejima and Sibaoka 1982; Hodick and Sievers 1988; Fromm and Spanswick 1993; Opritov et al. 2002).

Unlike other descriptions of electrical reactions in plants (Beilby 1982; Mummert and Gradmann 1991; Gradmann et al. 1993), our model takes into account the participation of Ca^{2+} in AP generation noted in a number of works (Davies 2006; Trebacz et al. 2006). Figures 3 and 4b show that the absence of extracellular Ca^{2+} suppresses AP generation by the model, which is in good agreement with the experimental data (Iijima and Sibaoka 1985; Hodick and Sievers 1988; Vodeneev et al. 2006; Felle and Zimmermann 2007) and supports the correctness of our model.

Thus, our results show that the elaborated model simulates the basic properties of AP generation in vascular plants; therefore, it can be used for subsequent analyses. Simulated kinetics of electrogenic ion fluxes before and during AP generation are shown in Fig. 5. K^+ and H^+ fluxes are basic at rest; Cl^- and Ca^{2+} fluxes increase at excitation. However, the increase of the Ca^{2+} flux is of short duration, and its magnitude remains two orders lower than that of K^+ , H^+ or Cl^- . This correlates well with an idea on about a regulatory role of Ca^{2+} during AP (Davies 2006; Trebacz et al. 2006; Felle and Zimmermann 2007). Changes of K^+ and Cl^- fluxes are of long duration and develop simultaneously. The latter possibly provides a significant efflux of these ions during AP: an increase of

Fig. 4 APs induced by gradual cooling. **a** Simulated AP; **b** simulated E_m change without Ca^{2+} in the apoplast; **c** experimental AP (Pyatygin et al. 1999b). Solid line, E_m changes; dashed line, changes of temperature



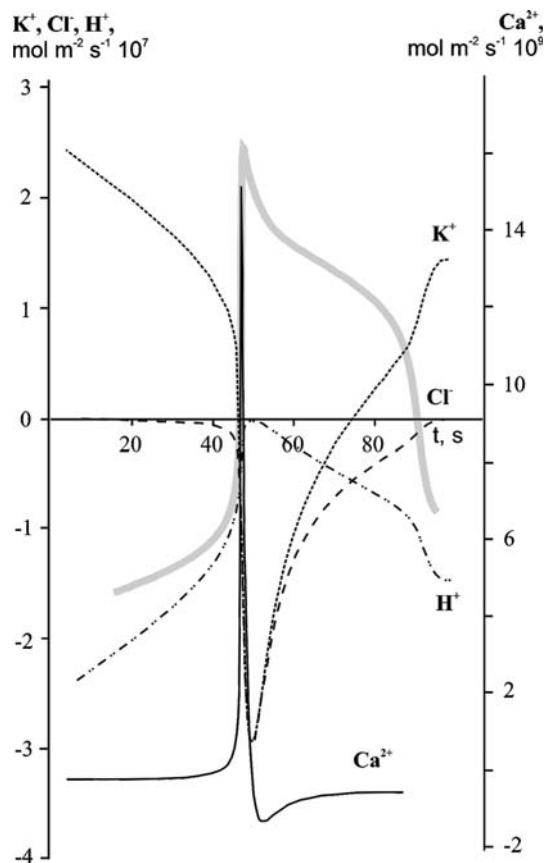


Fig. 5 Simulated kinetics of ion fluxes at AP generation. *Gray line* schematically shows the changes of E_m (without coordinate axis). +, influx; -, efflux

the Cl^- concentration in the apoplast is 2 mM and that of K^+ is 1.5 mM, which are close to the experimental data (Felle and Zimmermann 2007).

H^+ flux changes during AP generation are also shown in Fig. 5. The H^+ flux magnitude decreases at the AP depolarization phase. The development of the repolarization phase is connected with H^+ and K^+ fluxes at the first stage of repolarization (outward currents of K^+ and H^+) and only with the H^+ flux at the second stage, when E_m is more negative than the equilibrium potential for K^+ (the inward current of K^+ and the outward current of H^+). This result is in compliance with the hypothesis concerning H^+ -ATPase participation in AP generation.

The next stage of our investigation shows that a decrease of electrogenic pump activity induces depolarization of E_m and reduction of the simulated AP amplitude (Fig. 6a). AP does not develop if the portion of inactivated enzyme molecules is $>50\%$ (data not shown). This result is compatible with the influence of the H^+ -ATPase inhibitor DCCD on the electrogenesis of plant cells shown in Fig. 6b (Vodeneev et al. 2006). From that it follows that AP development requires some level of H^+ -ATPase activity.

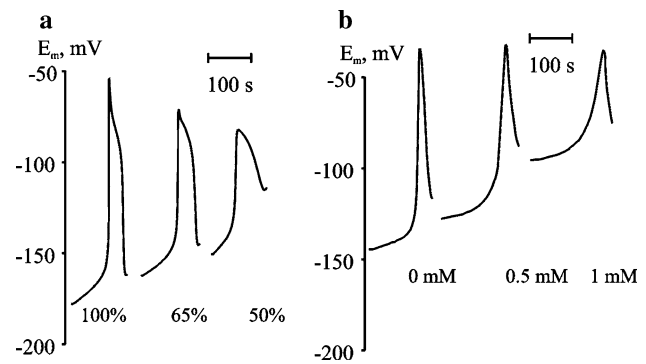
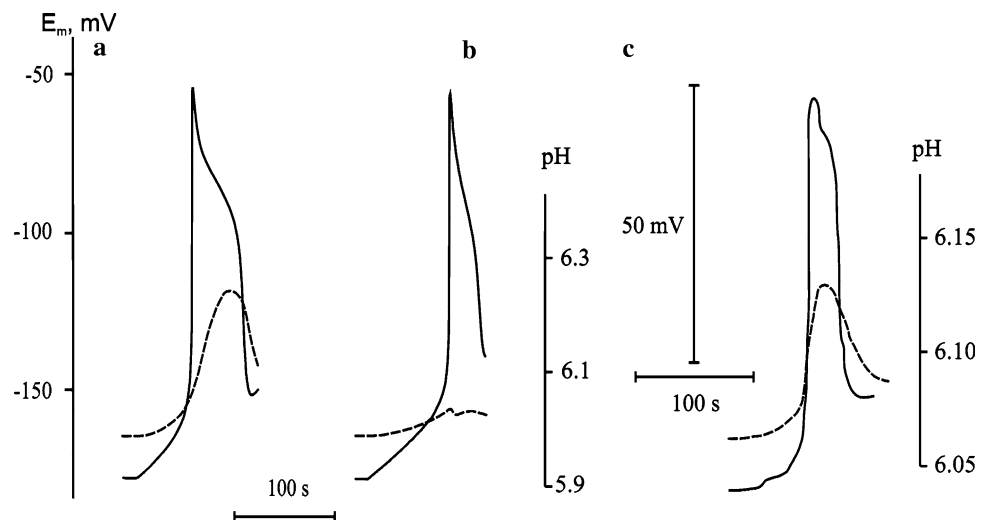


Fig. 6 Influence of H^+ -ATPase inactivation on the generation of cooling-induced APs. **a** Theoretical AP simulated by the model when H^+ -ATPase activity is equal to 100, 65 and 50% (inactivation magnitude shown in the figure). **b** Experimental AP (Vodeneev et al. 2006) measured when H^+ -ATPase activity is decreased by the inhibitor DCCD (concentrations shown in the figure)

To check the role of Ca^{2+} -dependent inactivation of H^+ -ATPase in AP generation, we studied the changes of pH in the apoplast since they are very important to support the participation of H^+ -ATPase in AP development (Vodeneev et al. 2006). Extracellular pH—simulated changes shown in Fig. 7a are in good qualitative agreement with the experimental curve (Fig. 7c). The quantitative differences are possibly related to the measurement of the surface potential and the registration of pH changes in the bathing solution (Vodeneev et al. 2006) since these methods cause a decrease of response magnitude. A connection of these changes with Ca^{2+} -dependent inactivation of H^+ -ATPase has been tested by a decrease of the enzyme activity dependence on calcium ions. The reduction of the Ca^{2+} influence on H^+ -ATPase is described as a decrease of the enzyme affinity for calcium ions (K for H^+ -ATPase is changed from 4×10^{-7} M to 10×10^{-7} M). The apoplast alkalization is suppressed in this case (Fig. 7b). This supports the necessity to describe the Ca^{2+} -dependent inactivation of H^+ -ATPase for the simulation of ion concentration changes (in particular, H^+ concentration) during AP. However, the development of the electrical response is not suppressed by the reduction of the Ca^{2+} influence on H^+ -ATPase. This result agrees with the experimental data, which show that a disruption of the Ca^{2+} influence on H^+ -ATPase suppresses the changes of H^+ concentration but weakly modifies the electrical reaction (Vodeneev et al. 2006). It demonstrates that the Ca^{2+} -dependent inactivation of the electrogenic pump is not strictly required for AP generation. The reduction of the Ca^{2+} -dependent inactivation of H^+ -ATPase can possibly stimulate activation of Cl^- channels, but this idea needs checking.

Thus, the elaborated model describes well the AP generation induced by external stimuli (electrical stimulation and gradual cooling) in vascular plants. The model analysis

Fig. 7 Changes in extracellular pH at cooling-induced AP generation. **a, b** Simulated changes in E_m and apoplastic pH at the high (**a**, $K = 4 \times 10^{-7}$ M) and low (**b**, $K = 10 \times 10^{-7}$ M) affinity of H^+ -ATPase for Ca^{2+} . **c** Experimental changes in the surface potential and pH in the extracellular solution (Vodenev et al. 2006)



has shown the activity of H^+ -ATPase to be a necessary condition for AP generation. Ca^{2+} influx induces the changes of this activity (inactivation and reactivation); however, these changes are not strictly required for AP generation. The compliance of the simulated and experimental data confirms the correctness of the hypothesis on H^+ -ATPase participation in AP generation. The model, therefore, can be used for further investigation of AP mechanisms.

In conclusion, some directions of the model development should be noted. (1) Additional cell compartments could be taken into account: the vacuole, which is the main store of ions in mature plant cells and takes part in AP generation in *Chara* alga (Shimmen et al. 1994; Beilby 2007); chloroplasts, which are also associated with AP generation in different plants (Bulychev and Turovetsky 1983; Shimmen et al. 1994; Bulychev et al. 2004; Fromm and Lautner 2007), etc. These modifications of the model could be a tool for theoretical analysis of a functional role of AP; however, elaboration of these models is restricted by the lack of experimental data on a relation between AP generation and the processes in the organelles of vascular plant cells. (2) Ensembles of a number of interrelated individual AP models can be elaborated. This system can be used to analyze AP propagation.

Acknowledgment This work was supported by the Russian Foundation for Basic Research (grant 09-04-01413-a).

References

Barbier-Brygoo H, Vinauger M, Colcombet J, Ephritikhine G, Frachisse J-M, Maurel C (2000) Anion channels in higher plants: functional characterization, molecular structure and physiological role. *Biochim Biophys Acta* 1465:199–218

- Beilby MJ (1982) Cl^- channels in *Chara*. *Phil Trans R Soc London B* 299:435–445
- Beilby MJ (2007) Action potential in charophytes. *Int Rev Cytol* 257:43–82
- Beilby MJ, Shepherd VA (2001) Modeling the current–voltage characteristics of charophyte membranes. II. The effect of salinity on membranes of *Lamprothamnium papulosum*. *J Membr Biol* 181:77–89
- Bentrup F-W (1990) Potassium ion channels in the plasmalemma. *Physiol Plant* 79:705–711
- Berestovsky GN, Kataev AA (2005) Voltage-gated calcium and Ca^{2+} -activated chloride channels and Ca^{2+} transients: voltage-clamp studies of perfused and intact cells of *Chara*. *Eur Biophys J* 34:973–986
- Blatt MR (1992) K^+ channels of stomatal guard cells. *J Gen Physiol* 99:615–644
- Bulychev AA, Turovetsky VB (1983) Light-triggered changes of membrane potential in the cells of *Anthoceros punctatus* and their relation to activation of chloroplast ATPase. *J Exp Bot* 34:1181–1188
- Bulychev AA, Kamzolkina NA, Luengviriya J, Rubin AB, Muller CS (2004) Effect of a single excitation stimulus on photosynthetic activity and light-dependent pH banding in *Chara* cells. *J Membr Biol* 202:11–19
- Bush DS (1993) Regulation of cytosolic calcium in plants. *Plant Physiol* 103:7–13
- Davies E (2006) Electrical signals in plants: facts and hypotheses. In: Volkov AG (ed) *Plant electrophysiology. Theory and methods*. Springer, Berlin, pp 407–422
- Felle HH, Zimmermann MR (2007) Systemic signaling in barley through action potentials. *Planta* 226:203–214
- Fromm J, Bauer T (1994) Action potentials in maize sieve tubes change phloem translocation. *J Exp Bot* 45:463–469
- Fromm J, Lautner S (2007) Electrical signals and their physiological significance in plants. *Plant Cell Environ* 30:249–257
- Fromm J, Spanswick R (1993) Characteristics of action potentials in willow (*Salix viminalis* L.). *J Exp Bot* 44:1119–1125
- Gradmann D (1976) “Metabolic” action potentials in *Acetabularia*. *J Membr Biol* 29:23–45
- Gradmann D (2001a) Models for oscillations in plants. *Aust J Plant Physiol* 28:577–590
- Gradmann D (2001b) Impact of apoplast volume on ionic relations in plant cells. *J Membr Biol* 184:61–69

- Gradmann D, Hoffstadt J (1998) Electrocoupling of ion transporters in plants: interaction with internal ion concentrations. *J Membr Biol* 166:51–59
- Gradmann D, Blatt MR, Thiel G (1993) Electrocoupling of ion transporters in plants. *J Membr Biol* 136:327–332
- Hansen U-P, Gradmann D, Sanders D, Slayman CL (1981) Interpretation of current-voltage relationships for “active” ion transport systems: I. Steady-state reaction-kinetic analysis of class-I mechanisms. *J Membr Biol* 63:165–190
- Hodick D, Sievers A (1988) The action potential of *Dionaea muscipula* Ellis. *Planta* 174:8–18
- Huang JW, Grunes DL, Kochian LV (1994) Voltage-dependent Ca^{2+} influx into right-side-out plasma membrane vesicles isolated from wheat roots: characterization of a putative Ca^{2+} channel. *Proc Natl Acad Sci USA* 91:3473–3477
- Iijima T, Sibaoka T (1985) Membrane potentials in excitable cells of *Aldrovanda vesiculosa* trap-lobes. *Plant Cell Physiol* 26:1–13
- Kinoshita T, Nishimura M, Shimazaki K (1995) Cytosolic concentration of Ca^{2+} regulates the plasma membrane H^{+} -ATPase in guard cells of fava bean. *Plant Cell* 7:1333–1342
- Klusener B, Weiler EW (1999) A calcium-selective channel from root-tip endomembranes of garden cress. *Plant Physiol* 119:1399–1405
- Lewis BD, Karlin-Neumann G, Davis RW, Spalding EP (1997) Ca^{2+} -activated anion channels and membrane depolarizations induced by blue light and cold in *Arabidopsis* seedlings. *Plant Physiol* 114:1324–1327
- Moran M, Ehrenstein G, Iwasa K, Mischke C, Bare C, Satter RL (1988) Potassium channels in motor cells of *Samanea saman*. A patch-clamp study. *Plant Physiol* 88:643–648
- Mummert H, Gradmann D (1991) Action potentials in *Acetabularia*: measurement and simulation of voltage-gated fluxes. *J Membr Biol* 124:265–273
- Opritov VA, Retivin VG (1982) On the mechanism of propagating excitation in higher plants. *Fiziol Rast* 29:915–924
- Opritov VA, Pyatygin SS, Retivin VG (1991) Bioelectrogenesis in higher plants [in Russian]. Nauka, Moscow
- Opritov VA, Pyatygin SS, Vodeneev VA (2002) Direct coupling of action potential generation in cells of a higher plant (*Cucurbita pepo*) with the operation of an electrogenic pump. *Russ J Plant Physiol* 49:142–147
- Pei Z-M, Baizabal-Aguirre VM, Allen GJ, Schroeder JL (1998) A transient outward-rectifying K^{+} channel current down-regulated by cytosolic Ca^{2+} in *Arabidopsis thaliana* guard cells. *Proc Natl Acad Sci USA* 95:6548–6553
- Pyatygin SS, Opritov VA, Khudyakhov VA (1992) Subthreshold changes in excitable membranes of *Cucurbita pepo* L. stem cells during cooling-induced action potential generation. *Planta* 186:161–165
- Pyatygin SS, Opritov VA, Abramova NN, Vodeneev VA (1999a) Primary bioelectric response of higher plant cells to the combined action of stress factors. *Russ J Plant Physiol* 46:530–536
- Pyatygin SS, Opritov VA, Polovinkin AV, Vodeneev VA (1999b) Mechanism of generation of action potential in higher plants. *Dokl Biophys* 364–366:42–45
- Pyatygin SS, Opritov VA, Vodeneev VA (2008) Signaling role of action potential in higher plants. *Russ J Plant Physiol* 55:312–319
- Rodriguez-Navarro A (2000) Potassium transport in fungi and plants. *Biochim Biophys Acta* 1469:1–30
- Samejima M, Sibaoka T (1982) Identification of the excitable cells in the petiole of *Mimosa pudica* by intracellular injection of procion yellow. *Plant Cell Physiol* 24:33–39
- Shimmen T, Mimura T, Kikuyama M, Tazawa M (1994) Characean cells as a tool for studying electrophysiological characteristics of plant cell. *Cell Struct Funct* 19:263–278
- Sukhov VS, Vodeneev VA (2005) Mathematical model of action potential in higher plant. In: Riznichenko GY (ed) Mathematics, computing, education [in Russian]. Regular and chaotic dynamics, Moscow-Izhevsk, pp 267–278
- Thomine S, Zimmermann S, Guern J, Barbier-Brygoo H (1995) ATP-dependent regulation of an anion channel at the plasma membrane of protoplasts from epidermal cells of *Arabidopsis* hypocotyls. *Plant Cell* 7:2091–2100
- Trebacz K, Dziubinska H, Krol E (2006) Electrical signals in long-distance communication in plants. In: Baluska F, Mancuso S, Volkmann D (eds) Communication in plants. Neuronal aspects of plant life. Springer, Berlin, pp 277–290
- Tyerman SD, Beilby M, Whittington J, Juswono U, Neyman L, Shabala S (2001) Oscillations in proton transport revealed from simultaneous measurements of net current and net proton fluxes from isolated root protoplasts: MIFE meets patch-clamp. *Aust J Plant Physiol* 28:591–604
- Vodeneev VA, Opritov VA, Pyatygin SS (2006) Reversible changes of extracellular pH during action potential generation in a higher plant *Cucurbita pepo*. *Russ J Plant Physiol* 53:481–487
- White PJ (1998) Calcium channels in the plasma membrane of root cells. *Ann Bot* 81:173–183
- Zawadzki T, Davies E, Dziubinska H, Trebacz K (1991) Characteristics of action potential in *Helianthus annuus*. *Physiol Plant* 83:601–604
- Zimmermann S, Sentenac H (1999) Plant ion channels: from molecular structures to physiological functions. *Curr Opin Plant Biol* 2:477–482