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Hodgkin-Huxley Analysis of Whole-Cell Outward Rectifying K^+-Currents in Protoplasts from Tobacco Cell Suspension Cultures

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Summary. The voltage and time dependence of outward-rectifying K^+ currents ($I_{K,out}$) measured in protoplasts from tobacco cell suspension cultures in the whole-cell configuration of the patch-clamp technique are quantitatively analyzed. The voltage and time dependence was described according to the Hodgkin and Huxley model for $I_{K,out}$ currents in the squid giant axon, and to allow comparison, in analogy with the quantitative analysis of $I_{K,out}$ currents in *Vicia faba* guard cell protoplasts as described by Schroeder (*J. Membrane Biol.*, **107**:229–235, 1989). The $I_{K,out}$ from tobacco could be described by a similar model as the $I_{K,out}$ from guard cell protoplasts (i.e., sigmoid activation time course, activation variable raised to second power, single exponential deactivating tail currents, absence of inactivation). However, in contrast to guard cells, both the activation and deactivation time constants were strongly voltage dependent in tobacco protoplasts. The voltage dependence of the transition rates for channel opening and channel closing was slightly asymmetrical and inverse to the asymmetry found in guard cells. The data presented show that the voltage-dependent kinetic properties of the $I_{K,out}$ conductance of tobacco protoplasts are different from these properties in guard cell protoplasts. This analysis provides a basis for the study of $I_{K,out}$ conductance function and modulation.

Key Words *Nicotiana tabacum* · ion channel · channel kinetics · patch clamp · outward rectifier

Introduction

The application of the patch-clamp technique to plant cell protoplasts has revealed the existence of different ion conductances in cells from different plant species and from different plant tissues. In most protoplasts studied a voltage-gated outward-rectifying K^+-selective current ($I_{K,out}$) is present (e.g., Schroeder, Hedrich & Fernandez, 1984; Schroeder, Raschke & Neher, 1987; Moran, Fox & Satter, 1990; Colombo & Cerana, 1991; Fairley, Laver & Walker, 1991; Ketchum & Poole, 1991; Schachtman, Tyerman & Terry, 1991; Fairley-Grenot & Assmann, 1992; Van Duijn et al., 1992; Van Duijn, Ypey & Libbenga, 1993). In all cases reported, the $I_{K,out}$ is depolarization activated, shows a sigmoidal activation time course, an exponential-like deactivation time course and lacks inactivation. However, diversity of $I_{K,out}$ in different species and tissues has only received limited attention (e.g., Fairley-Grenot & Assmann, 1992), and a detailed quantitative analysis is only available for the $I_{K,out}$ of guard cell protoplasts from *Vicia faba* (Schroeder, 1989). It is likely that the presence of different types of ion channels varies between different cell types and different species, which may be cell function related (e.g., ion channels involved in guard cell function may be absent in root cells). Therefore, exact knowledge about the presence of different types of ion channels in different types of cells (which requires comparison of ion channel properties in different cell types) may be of great help to understand ion channel function and developmental regulation of ion channel production. A detailed quantitative analysis is essential for comparison of $I_{K,out}$ conductance kinetics and for comparison of the regulation of $I_{K,out}$ conductance in different types of cells. In addition, the study of possible modulation of $I_{K,out}$ properties by cytoplasmic components or $I_{K,out}$ involvement in hormone signal transduction pathways requires a detailed quantitative knowledge of the kinetics of the conductance. Furthermore, such an analysis may provide a basis for understanding diverse functions of the $I_{K,out}$ in the different types of plant cells.

The $I_{K,out}$ likely plays an important role as a K^+ release channel during stomata closure in guard cells

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(Schroeder, et al., 1984; Schroeder, 1988), and during leaf movements (Moran et al., 1988). So far, no clear physiological function has been assigned to the $I_{K,out}$ in other cell types. The $I_{K,out}$ is present in protoplasts from tobacco cell suspension cultures (Van Duijn et al., 1992; Van Duijn, Ypey & Libbenga, 1993). These cells are undifferentiated and show expression of specific genes (e.g., Van der Zaal, Mennes & Libbenga, 1987) and membrane hyperpolarization (*unpublished results*) upon stimulation with the plant hormone auxin. Cells from these suspensions are used to study auxin signal transduction towards gene expression with molecular biological, biochemical and biophysical approaches (e.g., Van der Zaal et al., 1987; Mennes et al., 1992). The plant hormone auxin is essential in plant cell differentiation, cell division and cell elongation. Stimulation of plant cells with auxin induces fluxes of different ions (H^+, K^+, Ca^{2+}, Cl^-), which may be involved in the auxin signal transduction (e.g., Felle, 1988; Felle, Peters & Palme, 1991; Gehring, Irving & Parish, 1990; Marten, Lohse & Hedrich, 1991) or which may reflect hormone-controlled metabolic activity. The auxin-induced ion fluxes suggest the involvement of ion channels and/or ion pumps in the auxin signal transduction.

The patch-clamp technique (Hamill et al., 1981) allows the measurement of ionic currents in single cells under voltage-clamp conditions and, therefore, provides the possibility of investigating the involvement of ion channels in plant hormone signal transduction pathways. In first instance, this requires detailed knowledge of the ion conductances present in the unstimulated cells. In this report, the time and voltage dependency of $I_{K,out}$ currents of tobacco protoplasts is quantitatively analyzed using a Hodgkin-Huxley approach, in a similar way as described by Schroeder (1989). The properties of the $I_{K,out}$ from undifferentiated tobacco protoplasts are compared with the properties of the $I_{K,out}$ from the highly specialized guard cell protoplasts from *Vicia faba* leaves (Schroeder, 1989) and corn cell suspension protoplasts (Fairley, et al., 1991). It is concluded that, although the $I_{K,out}$ of tobacco cell suspension protoplasts and *Vicia faba* guard cell protoplasts can be described with the same type of Hodgkin-Huxley model, the voltage-dependent kinetic properties of the $I_{K,out}$ from these two cell types are completely different. This suggests that the $I_{K,out}$ channels in *Vicia faba* guard cell protoplasts are different from the $I_{K,out}$ channels in tobacco cell suspension protoplasts. The present analysis provides a firm biophysical basis for the study of $I_{K,out}$ function and possible involvement in plant hormone signal transduction.

Materials and Methods

Cell Cultures and Protoplast Isolation

Nicotiana tabacum L. (cv Bright Yellow) suspension cells were grown in a Linsmaier and Skoog medium (Linsmaier & Skoog, 1965) supplemented with aneurine-HCl (0.6 $mg.L^{-1}$) and 180 $mg.L^{-1}KH_2PO_4$ in the presence of 8.8×10^{-7} M 2,4-dichlorophenoxyacetic acid (2,4D) at 25°C in the dark according to An (1985). Protoplasts were prepared from cells in the stationary phase, i.e., seven days after transfer to fresh medium. The protoplasts were prepared and treated as described elsewhere (Van Duijn et al., 1992; Van Duijn, Ypey & Libbenga, 1993). Only protoplasts which were firmly attached to the glass coverslip in the measurement dish were chosen for experiments. Experiments were performed at room temperature (23°C). Protoplasts used for experiments had an average surface area of $2.9 \times 10^{-9} \pm 1.8 \times 10^{-9}$ m^2 ($n = 30$).

Solutions

Protoplasts were bathed in a standard extracellular solution (ECS) which was composed of (mM) 10 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 1 KOH, 10 MES (pH 5.5). The osmolarity of ECS was adjusted to about 1,010 mOsm or 570 mOsm with mannitol. The intracellular solution (ICS, i.e., pipette filling) consisted of (mM) 100 K-gluconate, 2 $MgCl_2$, 1.1 EGTA, 0.45 $CaCl_2$, 4 MgATP, 6 KOH, 10 HEPES (pH 7.0). The osmolarity of ICS was adjusted to about 708 mOsm with mannitol.

Patch-Clamp Experiments

The whole-cell configuration of the patch-clamp technique was applied to tobacco protoplasts with an L/M EPC-7 patch-clamp amplifier (List Electronics, Darmstadt, FRG) using conventional patch-clamp technique (Hamill et al., 1981). Borosilicate glass (GC150TF-15, Clark Electromedical Instruments, Reading, UK) patch-electrodes were used and had, after fire polishing, resistances ranging from 5 to 10 MΩ (measured in ECS). Giga-seals, and subsequently whole-cells, were made on tobacco protoplasts as described before (Van Duijn et al., 1992; Van Duijn, Ypey & Libbenga, 1993). The seal-resistances ranged between 2 and 10 GΩ.

The measured currents were low-pass filtered at 3 kHz before analog to digital conversion (ADC). The software package pCLAMP (version 5.5.1, Axon Instruments, Burlingame, CA) (Kegel et al., 1985) was used for the generation of sequences of test voltage potentials, data recording and data storage. The data were analyzed and fitted to different functions with the help of the software packages pCLAMP and FigP (version 6.0, Biosoft, Cambridge, UK).

Measured values are expressed as means ± SD, with n = number of cells measured, unless stated otherwise. Significance of differences in mean values was tested with Student's t-test ($P < 0.05$).

Results

In protoplasts from suspension cultures of a tobacco cell line, at least two different potassium selective ion conductances can be measured. All cells possess

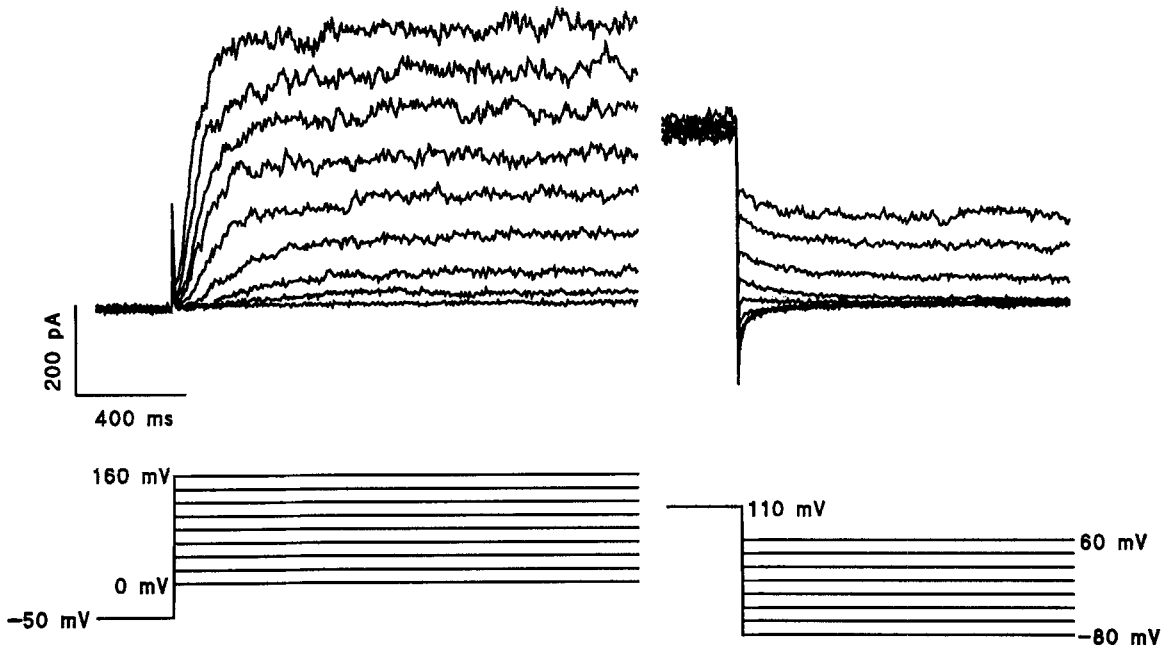

Fig. 1. Recordings of activation (upper left panel) and deactivation (upper right panel) of outward ($I_{K,out}$) K^+ currents across the plasma membrane of a single tobacco cell suspension culture protoplast in the whole-cell configuration of the patch-clamp technique. The extracellular solution contained 11 mM K^+ and the pipette solution (which equilibrates with the cytoplasm) contained 106 mM K^+. The upper left panel shows superimposed records of $I_{K,out}$ currents, activated upon nine different voltage steps to depolarizing potentials (ranging from 0 to +160 mV) from a holding potential of −50 mV, as illustrated in the voltage protocol (lower left panel). The upper right panel shows superimposed records of $I_{K,out}$ currents, deactivating upon eight different voltage steps to less positive potentials (ranging from +60 to −80 mV) from a $I_{K,out}$ activating potential of +110 mV, as illustrated in the voltage protocol (lower right panel). The voltage steps were made after the steady-state current was reached after a voltage step to +110 mV from a holding potential of −50 mV. All current traces shown are corrected for the leak conductance.

a relatively slowly activating outward current ($I_{K,out}$), while only about 30% of the cells shows the presence of an inward current as well (Van Duijn et al., 1992; Van Duijn, Ypey & Libbenga, 1993). In the current report, a detailed analysis of the kinetics of the $I_{K,out}$ is presented in terms of the Hodgkin and Huxley model for activation and deactivation of potassium currents in the squid axon (Hodgkin & Huxley, 1952), in analogy with the analysis of the $I_{K,out}$ current from *Vicia faba* guard cell protoplasts (Schroeder, 1989). Figure 1 shows the whole-cell current responses of a tobacco protoplast upon different step-wise voltage pulses of about 2.5 sec duration. Upon depolarization to potentials more positive than about 0 mV, the sigmoidal activating $I_{K,out}$ current is clearly visible. Exponential deactivation of the $I_{K,out}$ is visible in the decay of the currents upon stepping back to hyperpolarized potentials from a depolarized potential (Fig. 1).

Analysis of Activation of Outward K^+ Current

The $I_{K,out}$ reached a steady-state level within about 1 sec of depolarization (Fig. 1). There was no inactivation of this current (no reduction of maximal current during depolarization up to 20 sec, and maximal conductance was independent of holding potential (Van Duijn, Ypey & Libbenga, 1992)). Due to the absence of inactivation, the "real" maximal currents could be used in the analysis (in contrast to the frequently used peak currents for inactivating conductances). This simplified the analysis considerably.

In the squid giant axon an $I_{K,out}$ is present which shows sigmoidal and exponential deactivation (Hodgkin & Huxley, 1952). A mathematical description and physical interpretation of the $I_{K,out}$ kinetics of these axons was first described by Hodgkin and Huxley (1952). The current could be described by the following general equation:

$$I_K = n^p I_{K,max} \quad (1)$$

where n is the voltage and time dependent activation variable and $I_{K,max}$ is the maximal (completely activated) current (Hodgkin & Huxley, 1952). Potassium ions can only cross the membrane when p similar particles occupy a certain region of the mem-

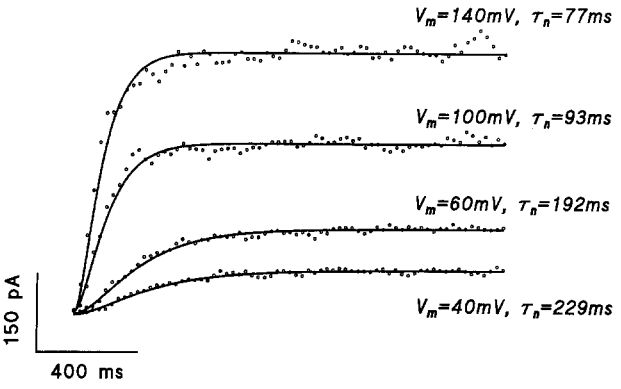

Fig. 2. $I_{K,out}$ current responses to four different potential steps to depolarized values (as indicated at each response) from a holding potential of −50 mV (open dots). One dot represents the mean values of four sample points taken around this time point. The Hodgkin-Huxley equation (Eq. (2)) with a parameter $p = 2$ was fitted to the current traces. The continuous lines show the fitted curves for the τ_n values indicated close to it. The current values at $t = 0$ were excluded from the fitting procedure.

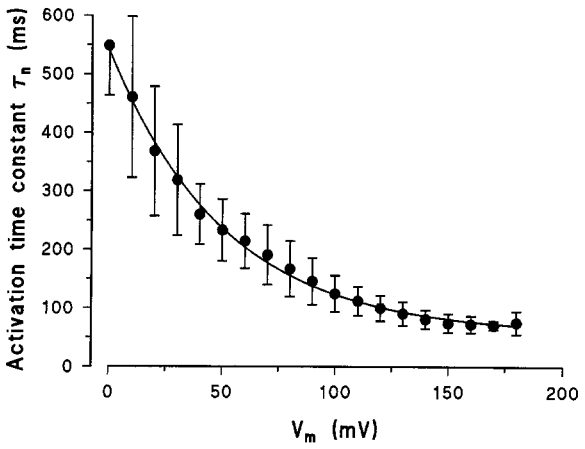

Fig. 3. Voltage dependence of the activation time constant τ_n obtained from fitting of Eq. (2) for $p = 2$ to current responses upon step-wise depolarization to different potentials from a holding potential of −50 mV, as shown in Fig. 2. Filled circles represent mean values obtained from 11 protoplasts, bars represent ± SD. The continuous line is the fitted curve according to Eq. (3).

brane, according to the interpretation of the value of p by Hodgkin and Huxley (1952). In other words, p can be interpreted as the number of independent membrane-bound gating particles (or gates), which control the opening of the channels. For p values larger than 1, I_k follows a sigmoidal time course of activation. Since the activation of the $I_{K,out}$ of tobacco protoplasts shows a sigmoidal time course (Fig. 1) the value of p will be larger than 1 for this conductance. The value of p was determined for $I_{K,out}$ of tobacco protoplasts by fitting the currents activated upon different step-wise depolarizations from a holding potential of −50 mV to the following equation:

$$I_{K,out} = I_L + I_{K,\infty}(1 - \exp(-t/\tau_n))^p. \quad (2)$$

In Eq. (2) I_L is the leakage current through the whole-cell membrane resistance (about 5 GΩ), $I_{K,\infty}$ is the steady-state activated current and τ_n is the activation time constant. Eq. (2) was fitted (with free running parameters $I_{K,\infty}$, τ_n and p) to the depolarization (0 to 140 mV) activated currents after leak correction. Care was taken to ensure that the fitted currents reached the steady state. Fitting with different models (one or two exponentials *vs.* sigmoidal) showed that fits with a sigmoidal model were superior. The mean value of p obtained for different currents from 10 protoplasts was 1.96 ± 0.52 (n = 37). This indicated that best fits could be obtained with a p value of approximately 2. For further analysis the value of p, therefore, was fixed at 2, which gives a good description of the activation of $I_{K,out}$ with Eq. (2) (Fig. 2).

The activation time constant τ_n (from Eq. (2)) was determined as a function of the membrane potential. Figure 3 shows that τ_n is clearly membrane potential dependent, with decreasing τ_n values for increasing membrane depolarization. The relationship between τ_n and V_m could be described by a single exponential function:

$$\tau_n = \tau_{n,min} + \tau_0 \exp(-V_m k) \quad (3)$$

where $\tau_{n,min}$ is the minimal τ_n, τ_0 is the value of $\tau_n - \tau_{n,min}$ at $V_m = 0$ mV, and k is a constant. Best fit was obtained for $\tau_{n,min} = 56.7$ msec, $\tau_0 = 485.4$ msec and $k = 0.020$ mV^{-1}.

Analysis of Deactivation of Outward K^+-Current

Deactivation of the $I_{K,out}$ can be seen in the tail currents upon stepping back to less positive potentials from strongly depolarized potentials (e.g., from +110 mV to values less positive than 60 mV) (Fig. 1). These tail currents followed an exponential time course and were fitted to sums of exponentials of the form:

$$I_{K,tail} = \sum I_{K,0} \exp(-t/\tau_n)^p \quad (4)$$

where τ_n is the deactivation time constant and $I_{K,0}$ is the initial current after a change of V_m. Figure 4 shows that, similar to the finding for $I_{K,out}$ in *Vicia faba* guard cells (Schroeder, 1989), $I_{K,tail}$ could be

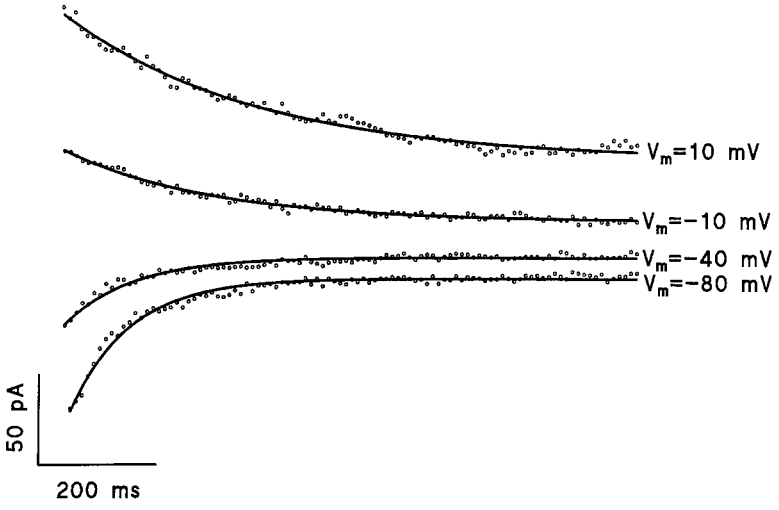

Fig. 4. Deactivation of $I_{K,out}$ currents upon four different hyperpolarizing voltage steps (as indicated by the V_m values to the right of each current trace) from a holding potential of +100 mV. Open dots represent the mean values (current and time) of two sample points. The continuous lines show the best single-exponential (Eq. (4)) fits to the data points. Time constants of deactivation, τ_n, obtained from these fits were: at V_m = 10 mV, τ_n = 655 msec; at V_m = −10 mV, τ_n = 496 msec; at V_m = −40 mV, τ_n = 228 msec; at V_m = −80 mV, τ_n = 198 msec. Current values at t = 0 were excluded from the fitting procedure.

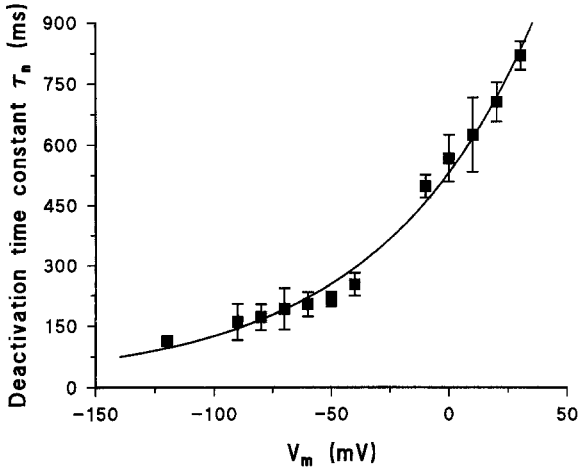

Fig. 5. Voltage dependence of the $I_{K,out}$ deactivation time constant, τ_n, obtained from fitting of Eq. (4) for p = 2 to current responses upon step-wise hyperpolarization to different potentials from a holding potential of +100 mV, as shown in Fig. 4. Filled squares represent mean values obtained from five protoplasts, bars represent ± SD. The continuous line is the fitted curve according to Eq. (3).

best described by a single exponential in most cases. τ_n was determined as a function of V_m in different cells. Figure 5 shows that τ_n is strongly voltage dependent and increases with increasing membrane depolarization. Due to the presence of only very small currents around the $I_{K,out}$ reversal potential no reliable τ_n values could be determined for these values of V_m. As for the τ_n of activation, the relationship between τ_n of deactivation and V_m could be described with a single exponential function (Eq. (3)). Best fit was obtained for $\tau_{n,min}$ = 14.2 msec, τ_0 = 517.3 msec and k = −0.015 mV^{-1}. From the used Hodgkin and Huxley model (Hodgkin & Huxley, 1952), it is expected that the value of τ_n of activation and deactivation must be equal for one potential value. In the presented experiments the τ_n of activation and deactivation become equal for a V_m around 0 mV.

Voltage-Dependent Steady-State Activation

The steady-state activation as a function of V_m of the $I_{K,out}$ was determined from the steady-state current responses on voltage pulses of 2.5 sec duration. The chord conductance (G_K) of the $I_{K,out}$ was calculated from the steady-state currents for different V_m values with respect to the reversal potential of the conductance (−30 mV). The steady-state value of the activation variable, n_∞, has for p = 2 the following relationship with the chord conductance according to the Hodgkin and Huxley (1952) model:

$$n_\infty^2 = G_K/G_{K,max} \tag{5}$$

where $G_{K,max}$ is the maximum chord conductance. In Fig. 6 the relationship between the mean n_∞^2 of 13 cells and V_m is shown. A Boltzmann distribution of similar form, as used by Schroeder (1989), was fitted to the mean values:

$$n_\infty^2 = \frac{1}{[1 + \exp((V_{0.5} - V_m)/S)]^2} \tag{6}$$

where $V_{0.5}$ is the potential value at which n_∞ = 0.5 and S is a slope factor. The best fit was obtained for $V_{0.5}$ = 28.1 ± 0.7 mV and S = 27.7 ± 0.6 mV.

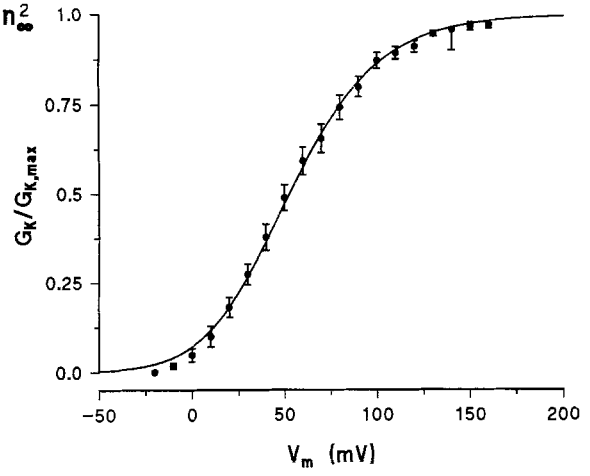

Fig. 6. Steady-state activation curve for $I_{K,out}$ conductance. n_∞^2 (activation degree) was calculated from $G_K/G_{K,min}$. Filled circles represent mean values obtained from 13 protoplasts, bars indicate ± SE. Continuous line shows the fitted Boltzmann distribution (Eq. (6)).

Channel Opening and Closing Kinetics

The voltage-dependent changes in the $I_{K,out}$ conductance are due to conformation changes in single ion channels. These channels show voltage-dependent transitions from closed states to an open state and *vice versa*. The kinetic scheme for these transitions in single ion channels can be deduced from the Hodgkin-Huxley model as described by Armstrong (1969). For the Hodgkin-Huxley model with a value of $p = 2$, the following, simplified, kinetic scheme is valid:

$$C_2 \underset{\beta_n}{\overset{2\alpha_n}{\rightleftharpoons}} C_1 \underset{2\beta_n}{\overset{\alpha_n}{\rightleftharpoons}} O \tag{7}$$

This three-state model has two closed states (C_1 and C_2) and one open state (O). In this scheme is α_n the voltage-dependent transition rate for channel opening and is β_n the voltage-dependent transition rate for channel closing. With the use of the measured values for n_∞ (Fig. 6) and the τ_n values for activation (Figs. 2, 3) and deactivation (Figs. 4, 5) the values of α_n and β_n could be calculated as a function of V_m with the equations described by Hodgkin and Huxley (1952):

$$\alpha_n = n_\infty/\tau_n \tag{8}$$

and

$$\beta_n = (1 - n_\infty)/\tau_n. \tag{9}$$

The calculated values of the transition rates α_n and β_n as a function of V_m are shown in Fig. 7. From Fig. 7 it can be seen that the voltage-dependent transition rates for channel opening and channel closing are equal at a membrane potential of about 20 mV. The voltage-dependent transition rates could be fitted with an equation of similar form as used by Hodgkin and Huxley (1952) for the outward potassium current in squid giant axon:

$$\alpha_n = \frac{\alpha_{n^0}(V_h - V_m)}{\exp\frac{V_h - V_m}{S_{\alpha_n}} - 1} \tag{10}$$

and

$$\beta_n = \frac{\beta_{n^0}(V_m - V_h)}{\exp\frac{V_m - V_h}{S_{\beta_n}} - 1} \tag{11}$$

in which α_{n^0} and β_{n^0} are scaling factors, V_h determines the voltage dependence and S_{α_n} and S_{β_n} determine the steepness of the voltage dependence of the rate constants α_n and β_n. The solid lines in Fig. 7 show the best fits. Best fit was obtained for α_n with $\alpha_{n^0} = 0.112 \pm 0.004\ mV^{-1}sec^{-1}$, $V_h = 35.8 \pm 3.8$ mV and $S_{\alpha_n} = 17.0 \pm 1.7$ mV. For β_n best fits were obtained with $\beta_{n^0} = 0.054 \pm 0.003\ mV^{-1}sec^{-1}$, $V_h = 28.7 \pm 5.7$ mV and and $S_{\beta_n} = 8.9 \pm 4.7$ mV. The resulting curves are somewhat asymmetrical (Fig. 7), but much less than reported for the $I_{K,out}$ of *Vicia faba* guard cells (Schroeder, 1989).

Discussion

A relatively slow sigmoidal-activating, noninactivating and exponential-deactivating outward potassium current has been indicated to exist in most plant protoplasts studied so far (e.g., Schroeder et al., 1984; Schroeder et al., 1987; Moran et al., 1990; Colombo & Cerana, 1991; Fairley et al., 1991; Ketchum & Poole, 1991; Schachtman et al., 1991; Fairley-Grenot & Assmann, 1992; Van Duijn et al., 1992; Van Duijn, Ypey & Libbenga, 1993). A shallow comparison of the $I_{K,out}$ from different types of protoplasts may lead to the conclusion that these conductances are identical. However, as already noted by Fairley-Grenot and Assmann (1992), more detailed comparisons indicate that differences in plant ion channels kinetics may be species dependent. A detailed comparison of the kinetics of the $I_{K,out}$ from

Table Summary of kinetic properties of $I_{K,out}$ currents in tobacco cell suspension protoplasts, *Vicia faba* guard cell protoplasts and corn shoot suspension protoplasts

	Tobacco cell suspension (this paper)	*Vicia faba* guard cell (Schroeder, 1989)	Corn shoot suspension (Fairley, Laver & Walker, 1991)
p	2	2	?
$V_{0.5}$	28 mV	−7 mV	around 0 mV
S	28 mV	21 mV	?
Activation time course	sigmoidal	sigmoidal[a]	sigmoidal
Activation τ_n V_m dependent	Yes (*cf.* Fig. 3) decreases as V_m increases	No very little	Yes decreases as V_m increases
Relative value of activation τ_n	(*cf.* Fig. 3)		
at +30 mV	slower	faster	slower
at +50 mV	equal	equal	slower
at +100 mV	faster	slower	slower
Deactivation time course	single exponential	single exponential	?
Deactivation τ_n V_m dependent	Yes (*cf.* Fig. 5) increases as V_m increases	Yes increases as V_m increases	?
Deactivation τ_n *vs.* V_m described by Eq. (3)			
$\tau_{n,min}$	14.2 msec	6.7 msec[b]	?
τ_0	517 msec	145 msec	?
k	−0.015 mV	−0.04 mV	?
Transition rates V_m dependent	(*cf.* Fig. 6)		
α_n	Yes	Little	?
β_n	Yes	Yes	?

[a] Recently Fairley-Grenot and Assmann showed that the activation of $I_{K,out}$ currents in *Vicia faba* guard cell protoplasts could be fitted better by a double-exponential function under their measurement and fitting conditions (Fairley-Grenot & Assmann, 1993).
[b] Eq. (3) was fitted to data from Schroeder (1989).

different protoplast types is difficult since an extensive quantitative analysis is not available for most of the published data. In addition, effects of plant hormones, second messengers, etc. on the $I_{K,out}$ may be only detectable and interpreted after a careful quantitative analysis.

In the present study a quantitative Hodgkin-Huxley analysis of the $I_{K,out}$ from tobacco protoplasts from cell suspension cultures was carried out according to the quantitative analysis of the $I_{K,out}$ of *Vicia faba* guard cells as described by Schroeder (1989). This quantitative analysis provides the possibility of comparing the kinetic properties of the $I_{K,out}$ from such different cells as tobacco cell suspension protoplasts and guard cell protoplasts.

The Table summarizes kinetic properties of $I_{K,out}$ in tobacco suspension culture protoplasts, *Vicia faba* guard cell protoplasts and corn shoot suspension protoplasts. The activation potential (as represented by $V_{0.5}$) varies considerably between these three cell types (Table). Different regulation of the activation potential and slightly different measurement conditions may (partly) explain these differences. For example, the activation potential of $I_{K,out}$ in corn root suspension protoplasts becomes more negative with an increase in cytoplasmic calcium

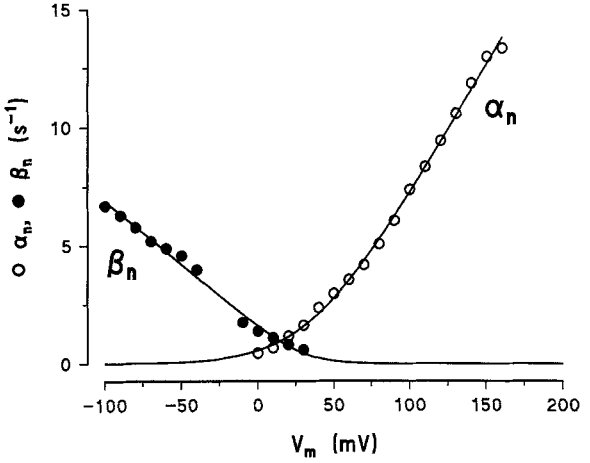

Fig. 7. Voltage dependence of transition rate for channel opening α_n (open symbols) and for channel closing β_n (filled symbols). The data points were fitted by Eq. (10) (for α_n) and Eq. (11) (for β_n) (continuous line).

concentration (Ketchum & Poole, 1991), whereas $V_{0.5}$ from guard cells is not very sensitive to cytoplasmic calcium concentration changes (Schroeder & Hagiwara, 1989; Fairley-Grenot & Assmann, 1992). From a comparison with the $I_{K,out}$ from other species and cell types at comparable cytoplasmic calcium concentrations (about 300 nM), it can be concluded that the half-maximal activation of the $I_{K,out}$ in tobacco suspension culture protoplasts is at relatively positive potentials.

As for the $I_{K,out}$ in *Vicia faba* guard cell protoplasts (Schroeder, 1989) the activation of the $I_{K,out}$ current from tobacco suspension protoplasts could be described accurately with the Hodgkin-Huxley model with a value of 2 for the power p of the activation parameter (Fig. 2). In addition, a single exponential description, as in *Vicia faba* guard cell protoplasts (Schroeder, 1989), fitted well with the deactivation (Fig. 4). These findings lead to the conclusion that the kinetics of the $I_{K,out}$ of tobacco suspension culture protoplasts can be described by a similar first-order reaction scheme as the $I_{K,out}$ from *Vicia faba* guard cell protoplasts (Schroeder, 1989). The proposed kinetic scheme, therefore, consists of two closed states and one open state (Eq. (7)). However, the voltage-dependent rates of channel opening (α_n) and channel closing (β_n) show a remarkable difference with the voltage-dependent opening and closing rates of $I_{K,out}$ in *Vicia faba* guard cell protoplasts (Schroeder, 1989).

The Table shows that large differences in both voltage dependence and relative value of the activation time constant can be found between different cell types. The activation is slowest in corn shoot suspension protoplasts. Like in tobacco suspension culture protoplasts and corn shoot suspension protoplasts, $I_{K,out}$ currents from animal cells generally show a strong voltage dependency of the activation time constant as well (e.g., Hodgkin & Huxley, 1952).

In both tobacco suspension culture protoplasts and *Vicia faba* guard cell protoplasts the deactivation time constant is strongly voltage dependent (Table), and the V_m dependency could be fitted by a single exponential function. The Table (parameter values from Eq. (3)) shows that the deactivation of $I_{K,out}$ in tobacco cell suspension protoplasts is much slower than in *Vicia faba* guard cell protoplasts.

The quantitative description of the channel opening rates (α_n) and channel closing rates (β_n) according to the Hodgkin-Huxley model very clearly shows the different kinetics of the $I_{K,out}$ in tobacco cell suspension protoplasts (Fig. 7) and *Vicia faba* guard cell protoplasts (Schroeder, 1989). The voltage dependence of α_n and β_n shows a slight asymmetry which is reflected in the difference of the values of α_{n^0} and β_{n^0} as well as the differences in the values of S_{α_n} and S_{β_n}. The observed asymmetry is, interestingly, inverse to the strong asymmetry found in *Vicia faba* guard cell protoplasts. The asymmetry found in the tobacco cell suspension protoplasts is similar to the asymmetry found in the squid giant axon (Hodgkin & Huxley, 1952). In addition, the voltage dependence of the transition rates could be fitted well with a similar equation as used for the $I_{K,out}$ of the squid giant axon (Fig. 7, Hodgkin & Huxley, 1952).

In summary it can be concluded that, although the $I_{K,out}$ currents of both tobacco cell suspension protoplasts and *Vicia faba* guard cell protoplasts can be precisely modeled with the same Hodgkin and Huxley model (activation power $p = 2$, and single-exponential deactivation) consisting of two closed states and one open state, the voltage dependence of the kinetic properties is very different (Table). The differences in the kinetic properties of the $I_{K,out}$ in different cell types may be related with different function in different cell types and/or species-specific differences. To test this hypothesis, measurements and quantitative analysis of $I_{K,out}$ currents from the same cell types in more different species (e.g., as done by Fairley-Grenot and Assmann (1992, 1993)), and from different cell types in the same species under precise identical conditions are necessary.

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