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Potassium Channels in Plasmalemma of *Nitella* Cells at Rest

A.I. Sokolik and V.M. Yurin
Laboratory of Photosynthesis, V.F. Kuprevich Institute of Experimental Botany, Byelorussian Academy of Sciences, Minsk, USSR

Summary. Cation channels of passive transport in the plasmalemma of *Nitella flexilis* cells at rest were studied by the voltage-clamp technique using microelectrodes. Two types of potassium channels have been identified. They are activated at different voltages: over −100 to −80 mV (D-channels) and below −100 mV (H-channels). The zero-current potential of instantaneous voltage-current curves (IVCC's) for both types of channels shifts by 50 to 55 mV in response to a 10-fold increase of K^+ concentration in the solution. Ion movement in D-channels follows the free diffusion mechanism; in H-channels the independence principle is violated. The channel selectivity (in the order of decreasing permeability) is: $K^+ > Rb^+ > NH_4^+ > Na^+ \geq Li^+ > Cs^+ > TEA^+ \approx$ choline$^+$. It has been found that D-channel Cs^+ block is potential dependent while tetraethylammonium (TEA^+) blocks H-channels in a potential-independent manner, but H^+ ions do not affect the inward potassium current of the channels. Two types of potassium channels appear to be located in different parts of the membrane and their entrance parts are of different structure.

Key Words *Nitella flexilis* · plasmalemma · potassium channels

Introduction

At present it is generally accepted that biological membranes possess specific sites (channels) through which ion transport takes place (Hille, 1970; Armstrong, 1975; and others). Excitable calcium-sodium and chloride channels (Volkov, 1977; Lunevsky et al., 1980; Berestovsky, 1981) have been identified in plant cell membranes. However, there are almost no systematic and reliable data on the properties of ionic channels of passive transport in plant cell membranes at rest. Taking into account the important physiological and potential-controlling functions of potassium ions at rest (Libbert, 1974; Hope & Walker, 1975; Yurin et al., 1977), we have attempted to identify and study the electrodiffusion properties of potassium channels in the plasmalemma of resting *Nitella* cells. Some experimental data discussed in this paper have been published earlier (Sokolik & Yurin, 1981).

Materials and Methods

The experiments were carried out in laboratory grown cells of fresh-water alga *Nitella flexilis*. The second or third internodal cells, 4 to 6 cm long and 0.3 to 0.4 mm in diameter, were selected. Two or three days before the experiment the cells were prepared, placed into artificial pond water (APW, 0.1 mM KCl + 1.0 mM NaCl + 0.2 mM $CaCl_2$) and kept in darkness. The experiments were run in darkness to prevent activation of the light-stimulated electrogenic pump (Spanswick, 1972; Bobrov, 1975; Lyalin, 1980).

Most of the experiments were made in a solution containing 5 mM K^+ ions. Potassium concentrations higher than that in APW considerably increased the potassium conductance fraction. Further increase in the potassium concentration can produce remarkable rearrangements of the membrane structure (Lucas, 1976, 1979). The chloride conductance fraction was somewhat decreased by using a less permeable anion SO_4^{2-} instead of Cl^- (Findlay et al., 1969). Contribution of calcium ions, present in the solution, to the conductance of resting membrane is negligible (Spanswick & Williams, 1965; Yurin et al., 1977).

At the initial stage of the experiment a cell was placed into APW, a microelectrode was inserted into the cell and then a solution with a high potassium ion concentration was let into the chamber. Concentrations of other ions depended on the experimental conditions. pH of the solution was adjusted by Tris-buffer or one of Good's buffers (MES) at 2 mM concentration each. Exchangeable calcium was removed from the cell wall using EDTA sodium salt. The ambient temperature was maintained constant at 20 ± 1°C.

Experimental Setup: Voltage-Current Characteristics of Plasmalemma

Conventional microelectrode technique was used for recording electrical characteristics of the membrane. The experiments were performed using voltage clamping on the plasmalemma of *Nitella flexilis* cells.

The tip of a 3 M KCl-filled microelectrode (Fig. 1) was inserted into the cell cytoplasm to measure the electrical properties of the plasmalemma. The experiment was carried out if the potential difference across the plasmalemma in the APW solution was close to potassium-equilibrium potential (−140 to −160 mV). The fact that the microelectrode tip is placed in the cytoplasm was determined by the cell response to a short-term illumi-

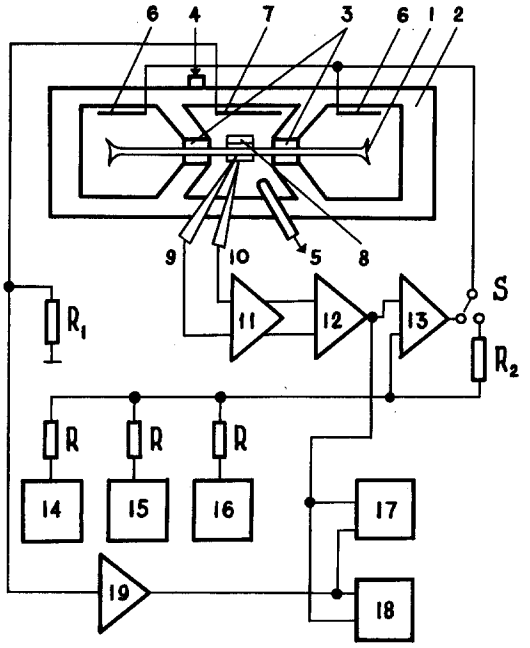


Fig. 1. Schematic diagram of experimental setup. 1—cell; 2—Plexiglas® chamber with three compartments; 3—isolating Vaseline®-filled gaps 10 mm wide; 4 and 5—inlet and outlet pipes for flowing the solution through the central compartment; 4-mm-long cell portion is in this compartment; rate of solution flow is 1 volume of central compartment per 10 sec; 6 and 7—Ag/AgCl current electrodes; 8—cell support; 9—microelectrode; 10—indifferent electrode; 11—probe voltage preamplifier, 10^{10} Ω input resistance; 12 and 13—operational amplifiers; 14, 15 and 16—control voltage generators; 14—steady voltage, 15—rectangular pulses; 16—sawtooth generator; 17—storage oscilloscope; 18—*X-Y* recorder. Gain factor with disconnected feedback is 10^3, time constant of the voltage-clamp system is 2 to 3 msec; *S*—voltage-clamping mode and current-clamping switch; R_1—current measuring resistor; R_2—resistor of about 10^8 Ω for current-clamping mode

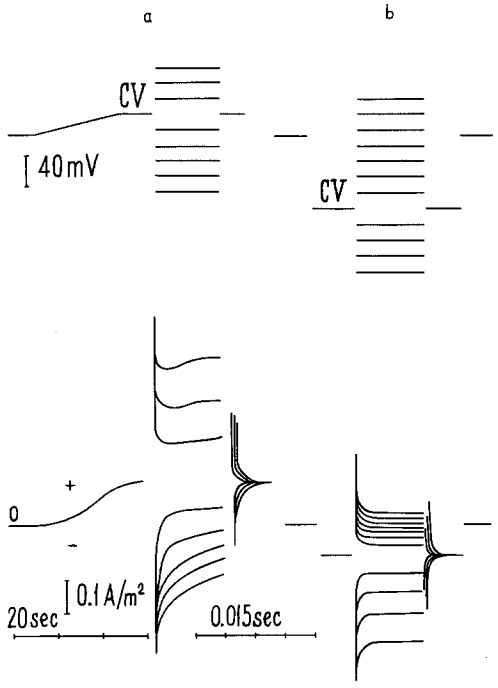


Fig. 2. The upper set of curves shows voltages applied to the plasmalemma to obtain the IVCC's, and the lower set the corresponding membrane currents. The resting potential difference is −70 mV. The outward current is positive. The inward current is negative. 2.5 mM K_2SO_4 + 0.2 mM $CaCl_2$ (i.e. 5 K^+) solution: (*a*) conditioning voltage (CV) of −50 mV; (*b*) CV of −170 mV. Current was sampled every 2 msec, the first sample taken 4 msec after the pulse origin

nation, by the type of action potential and resting potential difference (RPD) (Findlay & Hope, 1964; Bobrov et al., 1973).

Plasmalemma depolarization caused activation of fast potential-dependent calcium-sodium and chloride channels thus generating the action potential (Kitasato, 1973; Vostrikov, 1976; Volkov, 1977).

The effects which appear during the membrane excitation (for instance, calcium ion influx into cytoplasm) and affect the potassium conductance of the plasmalemma in a wide range of voltages were eliminated in the following way. As was mentioned above, a cell was placed into a solution containing a high concentration of potassium ions. The plasmalemma voltage was clamped at the resting potential level and then slowly, at a rate of about 0.2 to 0.5 mV/sec, it was shifted towards depolarization until the desired conditioning voltage (CV) was attained. At this rate of depolarization no rapid inward current was observed (Fig. 2). Apparently, membrane "adaptation" took place due to an equilibrium in the (chloride and calcium-sodium) channel activation-inactivation processes. The outward current considerably increased with the membrane depolarization and reached a steady level upon voltage stabilization. At this stage a train of pulses of different amplitude (test pulses) was applied to the plasmalemma and the resultant current curves were recorded (Fig. 2).

For obtaining instantaneous voltage-current curves (IVCC's) the shortest possible duration of the test pulse was taken, *ca.* 10 to 20 msec, so that the plasmalemma permeability changes were as small as possible in response to every pulse. The changes were monitored by the clamp current in the pulse-to-pulse intervals. The interval duration was selected so as to allow the plasmalemma to restore its conductance. The current pulse recorded on the oscilloscope screen consisted of a capacitance component (2 to 3 msec duration) and exponential component of the ionic current (Fig. 2). The instantaneous current value was obtained by extrapolating the ionic component to the pulse origin. The extrapolated current values were used to plot the IVCC's.

Steady voltage-current curves (SVCC's) were either plotted from the steady-state ionic current values during clamping of each successive voltage step, or were directly recorded with an *X-Y* recorder. In the latter instance the linearly changing voltage across the plasmalemma was clamped. To ensure steady VCC's the rate of voltage change was set to be reasonably low, about 0.2 to 0.5 mV/sec. A VCC was considered steady if there was no hysteresis during trace and retrace. In this case the control voltage pulses were triangular.

All calculations were performed on a digital computer. The experimental results were treated by conventional statistical methods (Hudson, 1964).

Results

The resting potential of plasmalemma in a solution containing 5 mM potassium ions decreased to −60 to −80 mV, and its mean value was −77 ± 5 mV. Typical current curves at various CV's and a family of IVCC's plotted from the current curves are shown in Figs. 2 and 3. First of all, it should be noted that the IVCC's are nonlinear. If the CV is changed over a wide range, the IVCC's essentially alter their shape, two basic types of curves being observed. On depolarization the curves take the Goldman shape. In the −40 to −20 mV range the IVCC's became essentially unchanged. As the CV decreased towards hyperpolarization, rectification of non-Goldman type was observed. Zero-current potential was almost the same for all IVCC's and conductance at this potential sharply decreased with plasmalemma hyperpolarization. Over the voltage range below −140 mV the IVCC's became unchangeable.

The changes in the IVCC shape and in the plasmalemma conductance over a wide range of voltages may be caused, apart from the potential dependence, by the concentration depletion in the solution layer adjacent to the membrane, provided by the inward current during hyperpolarization. Indeed, the transfer numbers for potassium ions in the membrane and in the solution are different. The potassium concentration in the solution is rather low (5 mM), the inward current at CV = −200 mV is approximately 0.1 A/m^2. Calculations in accordance with Lerch data (Lerch & Wolf, 1975*a*,*b*) showed that K^+ concentration in the solution layer adjacent to the membrane decreased by 1.5 mM at the most unfavorable assumptions. Obviously, such concentration changes cannot cause a 10-fold conductance decrease shown by the experiment (Fig. 3).

Thus, the IVCC changes prove that plasmalemma properties are potential dependent.

We can therefore identify two voltage ranges where the plasmalemma potassium conductance is characterized by IVCC's of different shape. This is caused, as is shown below, by two types of potassium channels. In the range above −100 to −80 mV they will be referred to as D-channels; in the range lower than −100 mV they will be called H-channels.

Both types of IVCC's proved to be insensitive to decyclohexylcarbodiimide (DCCD), a classic inhibitor of the electrogenic proton pump (Spanswick, 1974), even at a concentration of 2×10^{-4} M. It means that, in our experiments, there was no contribution of electrogenic active transport to the process under study.

When SO_4^{2-} and benzenesulfonate ($C_6H_5SO_3^-$) was substituted for Cl^-, the IVCC's were not affected. When 5 mM of Na_2SO_4 was added to the solution with 2.5 mM K_2SO_4 + 0.2 mM $CaCl_2$, the change of inward current was insignificant; moreover, the current tended to decrease (the reason will be discussed below). This shows low Na^+ permeability of the channels. Therefore, sodium ions were used to maintain a constant ionic strength of the solution to ensure a constant surface potential of the plasmalemma (Krawczyk, 1975; Sokolik, 1978). The above results suggest that the identified D- and H-channels of the plasmalemma are potassium channels and belong to the passive transport system.

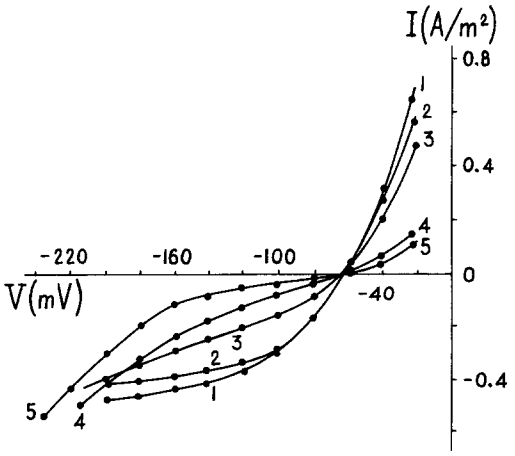


Fig. 3. Plasmalemma IVCC's obtained for 5 K^+ solution at different CV. Curves 1 to 5 have been obtained at CV's of −20, −40, −60, −100 and −180 mV, respectively

In order to get more detailed data on the response of IVCC's to various K^+ concentrations, a cell was placed in a solution containing 0.5 mM K_2SO_4 + 7 mM Na_2SO_4 + 0.2 mM $CaCl_2$, then sodium was substituted by potassium so that the total concentration of these cations remained the same. The IVCC's shape of D-channels did not essentially change (Fig. 4*a*): rectification of Goldman's type persisted, and only inward current increased. The IVCC's of H-channels changed significantly. As the K^+ concentration was increased, inward current across H-channels rose but to a smaller extent than in D-channels. In addition when K^+ concentration was decreased to 2 mM and lower, rectification tended to Goldman's type. When the potassium concentration was above 1 mM, both types of channels were highly selective to K^+: a 10-fold change of K^+ concentration shifted the zero-current potential for about 50 to 55 mV (Fig. 5).

One type of conductance gradually passed over to the other; an intermediate state was also observed, i.e. when both types of channels were open

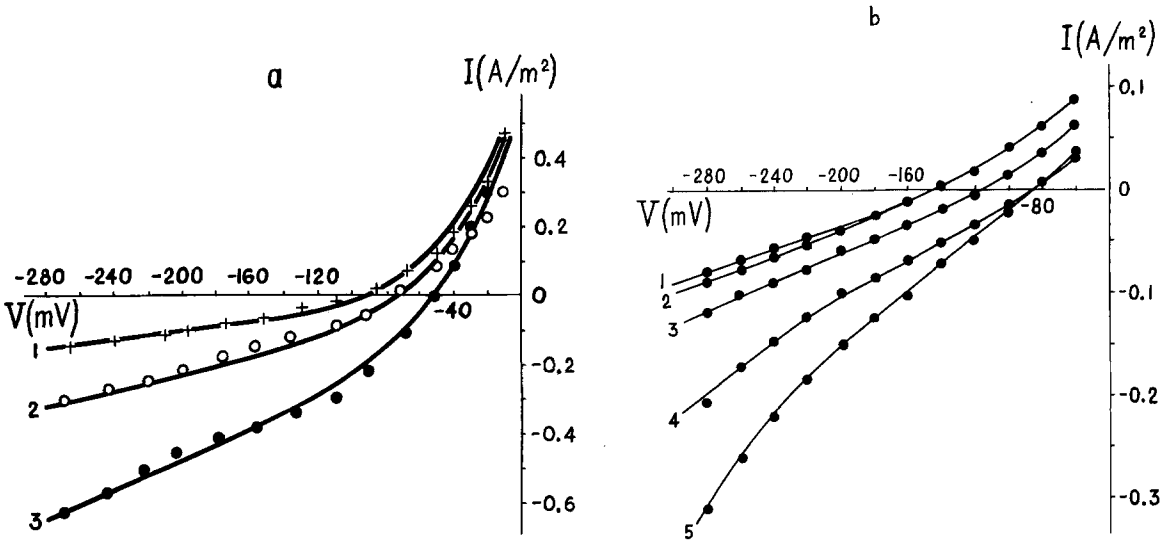


Fig. 4. (*a*) D-channel IVCC's obtained at varying K^+ concentration in the solution. Dependences represented by black circles, light circles and crosses have been obtained in the respective solutions: 5 mM K_2SO_4 + 2.5 mM Na_2SO_4 + 0.2 mM $CaCl_2$; 2.5 mM K_2SO_4 + 5 mM Na_2SO_4 + 0.2 mM $CaCl_2$; 1 mM K_2SO_4 + 6.5 mM Na_2SO_4 + 0.2 mM $CaCl_2$. CV is −40 mV. Curves 1, 2 and 3 have been drawn according to Eq. (1) with the following parameters: C^i = 79.3 mM; γ = 1.16, $P_K = 5.7 \times 10^{-6}$ cm/sec and K^+ concentration of 2.0, 5.0, 10.0 mM, respectively (*see* Discussion for details). (*b*) H-channel IVCC's (curves 1 to 5) obtained at K^+ concentrations of 0.5, 1.0, 2.0, 5.0, 10.0 mM. The initial solution contained 0.25 mM K_2SO_4 + 7.25 mM Na_2SO_4 + 0.2 mM $CaCl_2$; sodium was substituted for potassium with their total concentration remaining constant. CV = −200 mV

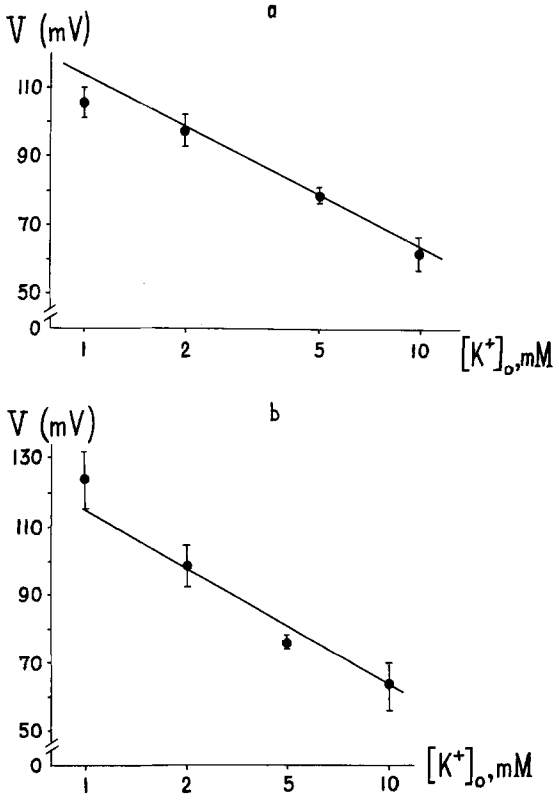


Fig. 5. Zero-current potential (for both types of channels) *vs.* K^+ concentration in the solution. Potassium was substituted for sodium with their total concentration remaining constant ($[K]_o$ + $[Na]_o$ = 10 mM). The lines have been drawn with a 50-mV slope for 10-fold K^+ concentration increase: (*a*) D-channels; CV = −40 mV; (*b*) H-channels; CV = −180 mV

(curves 3 and 4 in Fig. 3), and the IVCC's represented the sum of curves of the two above types. At the same time it is clear that in the depolarization region (CV was above −60 mV, curves 1 and 2 of Fig. 3) the IVCC changes primarily revealed D-channel activation. The IVCC shape was almost the same only the current amplitude increased: the ratio of currents in curves 1 and 2 was constant for all voltages. When CV shifted towards hyperpolarization, beginning from −100 mV (curve 4, Fig. 3), the IVCC shape changed as if H-channels were open and current across them prevailed over the current across D-channels. However, a further decrease of CV (curve 5, Fig. 3) did not result in a current rise, so H-channel activation was not obvious.

The above activation was observed under special conditions in spring-grown cells (March, April). Pretreated with 0.2 mM EDTA solution to remove exchangeable calcium, the cells were placed into 2.5 mM K_2SO_4 solution. pH of the solution was adjusted to 7.6 with 2.0 mM Tris-buffer and NaOH. When the potential difference across the plasmalemma of such a cell was changed at −200 mV for 1 to 3 min, D-channels closed and were not activated by voltage shifted to the depolarization region. In this case the plasmalemma conductance was associated with H-channels over the entire range of voltages and, as the CV was changed, the channels were activated in the range of −80 to −160 mV (Fig. 6). If the cell was returned to a solution with a higher content of Ca^{2+} (0.2 to 0.6 mM), D-channels restored their activation ability.

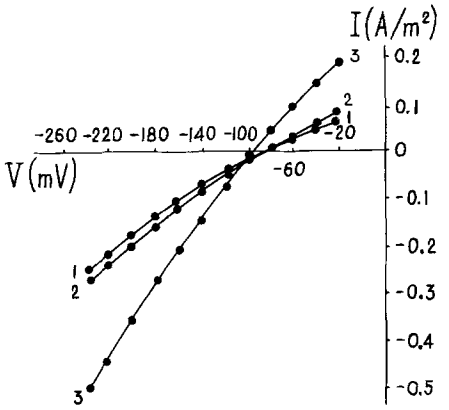


Fig. 6. H-channel IVCC's obtained under special conditions with D-channels inactivated. Details are in the text. Curves 1, 2 and 3 have been obtained in 2.5 mM K_2SO_4 + 0.01 mM $CaSO_4$ solution at CV equal to −40, −80 and −160 mV, respectively

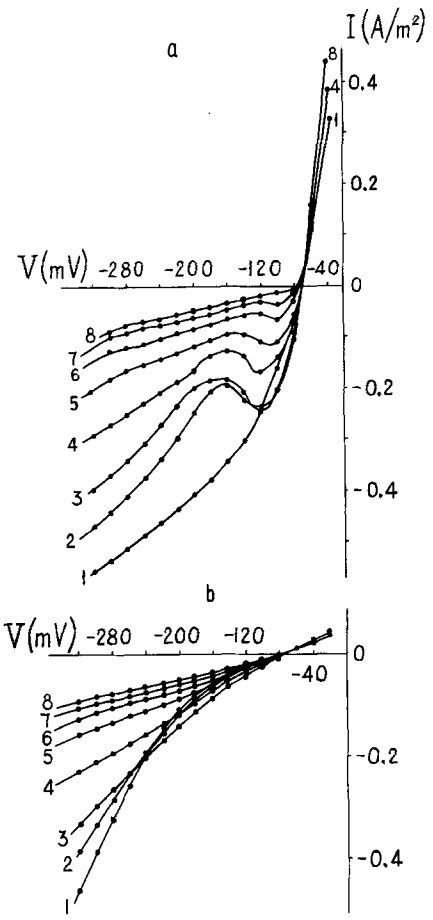


Fig. 7. D- (*a*) and H- (*b*) channel IVCC's obtained on addition of Cs^+ to 5 K^+ solution. CV was −50 and −180 mV, respectively. IVCC's (1 to 8) have been obtained at the following Cs^+ concentrations: 0, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 mM

The activation curve plotted from the data of Fig. 3 is S-shaped (*see* Fig. 13) as is typical for most of the ionic channels (Khodorov, 1975).

Similar to the potassium channels of animal cell membranes (Khodorov, 1975), D-channel activation and deactivation time course was exponential and time constants of these processes were potential dependent (Sokolik & Yurin, 1981).

During extended (2 to 3 min) depolarization of plasmalemma, the current reached its peak and then began to decline to attain a steady level. The conductance decrease was similar to that at the slow inactivation process in the potassium channels of animal cell membranes (Ehrenstein & Gilbert, 1966).

Cation-Induced IVCC Changes

The IVCC's of D- and H-channels in 5 mM solutions of K^+, Rb^+, NH_4^+, Cs^+, Li^+, Na^+, tetraethylammonium (TEA^+) and choline$^+$ were obtained.

The IVCC shape for both types of channels remained unchanged, the zero-current potential significantly shifted and inward current amplitude changed.

Cs^+, TEA^+ and H^+ Effect on IVCC

Cs^+ ions did not affect the time dependence of current across D- and H-channels; therefore blocking was completed for a time interval which was at least shorter than the current capacitance component (2 to 3 msec).

From 0.05 mM concentration of Cs^+ up, the inward current declined and a negative resistance branch appeared on the IVCC of D-channels (Fig. 7*a*). The inward currents in both types of channels decreased rapidly as Cs^+ concentration in the solution was increased: in the range of 2 to 5 mM Cs^+ the rate of current fall diminished. 5 mM Cs^+ inhibited 80 to 90% of D-channel inward current and up to 70% of H-channel inward current (Fig. 7*b*).

The outward current across H-channels was almost unchanged, while in D-channels the current increased by 20 to 30% in the presence of caesium. A slight shift of about 4 to 8 mV of zero-current potential towards hyperpolarization was observed in D-channels as Cs^+ concentration was increased. When the cells were kept in a solution containing 5 mM of Cs^+ for 2 or 3 hr, the IVCC's for both types of channels did not change. The Cs^+ blocking effect was completely reversible, i.e. in 5 to 10 min after the cells were placed in the initial solution the IVCC's restored their original shape.

Another "classical" blocker of potassium channels in animal cell membranes, TEA^+, also blocked the plasmalemma D- and H-channels. Figure 8 shows the IVCC's for both types of channels, obtained for TEA^+ concentrations ranging from 2 to

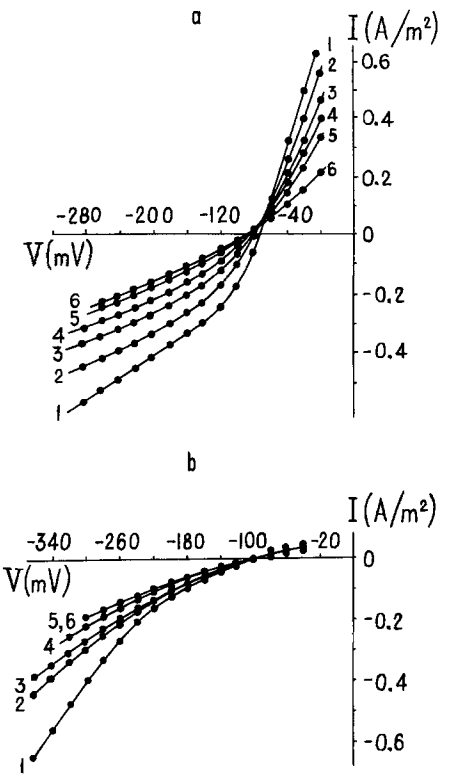


Fig. 8. D- and H-channel IVCC's obtained on addition of TEA^+ to 5 K^+ solution. (*a*) D-channels (CV = −40 mV). Curve 1 has been obtained for a TEA^+-free solution (control curve); 2 to 5—obtained at TEA^+ concentrations of 2.0, 6.0, 12.0, 24.0 mM; curve 6—obtained after 2-hr exposure in 24.0 mM TEA^+; (*b*) H-channels (CV = −180 mV). Curve 1 is the control curve; curves 2 to 5—obtained at 2.0, 6.0, 12.0 and 24.0 mM TEA^+, respectively; curve 6—obtained after 2-hr exposure in 24.0 mM TEA^+ solution

24 mM. It can be seen that TEA^+ ions effectively inhibited the potassium current in the channels of both types. The degree of inhibition enhanced monotonically as the TEA^+ concentration increased. For H-channels the TEA^+ blocking effect was potential dependent and similar to that of caesium (only the inward current was suppressed; *see* Fig. 9), whereas in D-channels TEA^+ inhibited both inward and outward currents.

The TEA^+ blocking effect reached its maximum for both types of channels 3 to 5 min after TEA^+ was added to the solution, i.e. within the period of time usually required for setting the electrophysiological characteristics of algae cell membranes following a change in the ionic composition of the medium. The potassium current time dependence due to channel activation or inactivation did not change in the presence of TEA^+.

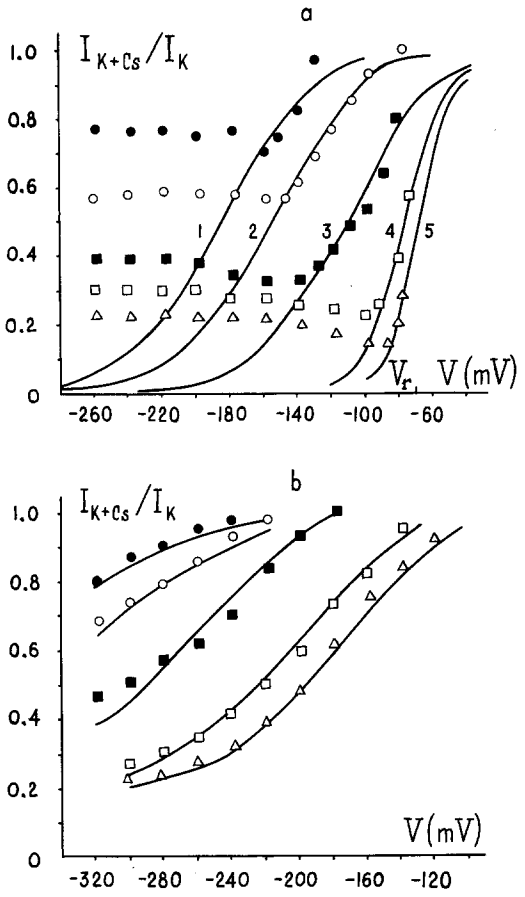
When the cells were kept in 24 mM TEA^+ solution for 2 hr the inward and outward currents of H-channels did not remarkably change (Fig. 8*b*, curves 5 and 6). At the same time the outward current in D-channels considerably decreased, while the inward current remained essentially unchanged (Fig. 8*a*). After the cells treated with TEA^+ for 2 hr were placed in the initial solution, the original IVCC's of H-channels were restored within 5 to 10 min. The inward current of D-channels was restored somewhat more slowly (within 15 to 20 min), while the outward current did not reach its initial level even after a 2-hr wash-out.

To study the effect of hydrogen ions on the potassium channel conductance, the cells were placed into a medium containing a high Ca^{2+} concentration (4 mM). Under such conditions the plasmalemma surface charges were almost fully compensated. The IVCC's were obtained at pH range from 5.1 to 8.6. In an acidic medium D-channel conductance decreased (Fig. 10). For H-channels no similar effect was observed. D-channels were blocked in a potential independent manner, although the block was not complete. When the blocking effect reached its steady level at pH equal to 5.5 to 6.0, 30 to 40% of the channel conductance remained unblocked (Fig. 11). A similar effect was obtained for the potassium channels of a squid axon membrane on varying the intracellular pH (Wanke et al., 1979). Using the model described in the above work we found the pK value of the proton-binding centers to be 7.1.

Ca^{2+} and H^+ Effect on Plasmalemma Surface Charges

It is well known that calcium and hydrogen ions play an important role in the control of ionic transport across the cytoplasmic membranes of plant cells (Shone et al., 1973; Clarkson, 1974; Luttge & Higinbotham, 1979). One of the possible mechanisms of this control is the interaction of these cations with fixed membrane surface charges.

In a solution containing 2.5 mM K_2SO_4 and 0.02 or 0.1 mM $CaSO_4$ pH was varied between 5 and 8. In the experiments on the effect of Ca^{2+} the solution contained 2.5 mM K_2SO_4 and 0.01 to 4.0 mM $CaSO_4$ at pH = 7.5. The IVCC's of D- and H-channels at the above concentrations of H^+ and Ca^{2+} did not alter their shape, and the zero-current potential remained the same. As the concentrations of both ions were decreased, the conductance of D-channels rose markedly. When the solution pH was decreased and Ca^{2+} concentration increased, the conductance activation curves of D-channels plotted from the SVCC's (Fig. 12) were shifted along the voltage axis towards the plasmalemma depolarization, and maximum conductance of D-channels de-



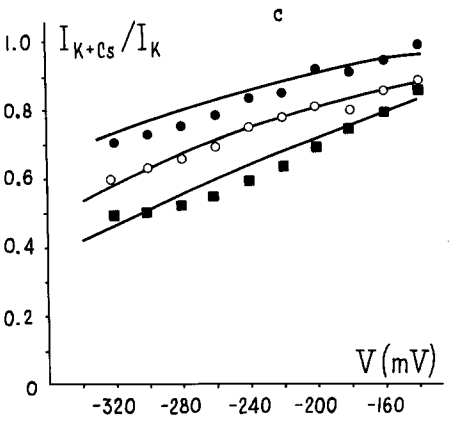


Fig. 9. Channel blocking (I_{K+Cs}/I_K) *vs.* membrane voltage. (*a*) Cs^+ block of D-channel. ●—0.05 mM Cs^+, ○—0.1 mM, ■—0.5 mM, □—1.0 mM, △—2.0 mM. The curves were drawn according to Eq. (3) with the following parameters: 1—δ = 0.90, K = 40 mM; 2—δ = 0.97, K = 40 mM; 3—δ = 0.98, K = 40 mM; 4—δ = 2.10, K = 1400 mM; 5—δ = 2.40, K = 1800 mM and corresponding Cs^+ concentrations. (*b*) Cs^+ block of H-channels. ●—0.05 mM Cs^+, ○—0.1 mM, ■—0.2 mM, □—1.0 mM, △—2.0 mM. The curves were drawn according to Eq. (3) with δ = 0.55, K = 100 mM and corresponding Cs^+ concentrations. (*c*) TEA^+ block of H-channels. ●—2 mM TEA^+, ○—6 mM, ■—12 mM. The curves were drawn according to Eq. (3) with δ = 0.24, K = 190 mM and corresponding TEA^+ concentrations

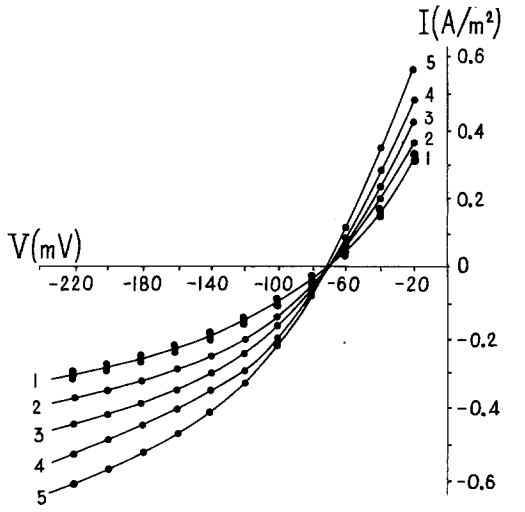


Fig. 10. D-channel IVCC's (CV = −30 mV) obtained for 2.5 mM K_2SO_4 + 4.0 mM $CaSO_4$ solution at the following pH values: 1—5.1, 5.5, 6.1; 2—6.6; 3—7.1; 4—7.7; 5—8.6

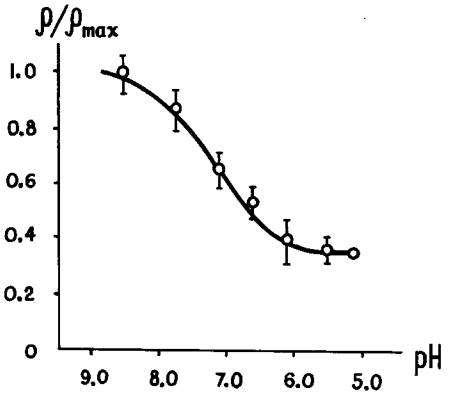


Fig. 11. H^+ block effect on D-channel conductance *vs.* solution pH. The curve has been drawn as a result of calculations in terms of the model described by Wanke et al. (1979)

creased significantly (Fig. 13). At the same time H-channel parameters did not alter.

It may be supposed that the shift of the activation curve along the voltage axis, measured by the change of voltage ($V_{1/2}$), at which the conductance is equal to half of its maximum value, is equal to the change of the surface potential (Mozhaeva & Naumov, 1972). The dependence of $V_{1/2}$ on the solution pH is illustrated by the curves in Fig. 14; a similar dependence of $V_{1/2}$ on Ca^{2+} concentration is close to linear and has a 15-mV slope.

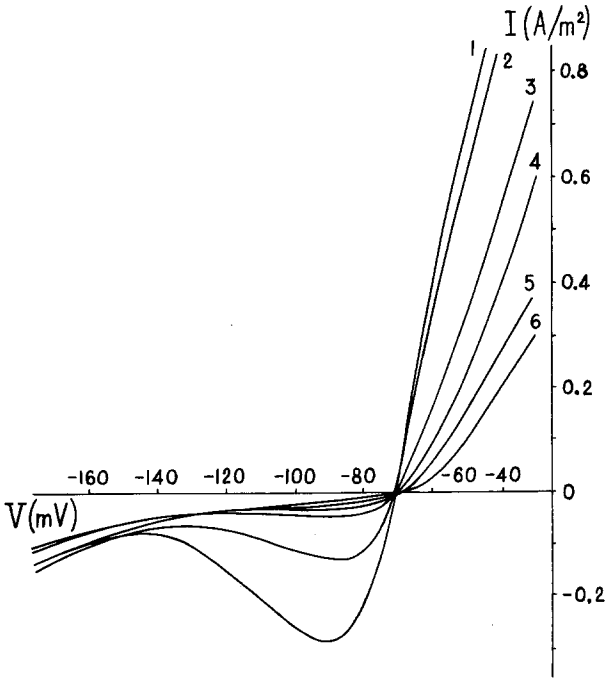


Fig. 12. Plasmalemma SVCC's in a solution containing 2.5 mM K_2SO_4 at various Ca^{2+} concentrations. Curves 1 to 6 correspond to 0.01, 0.03, 0.1, 0.3, 1.0, 4.0 mM $CaSO_4$ in the solution

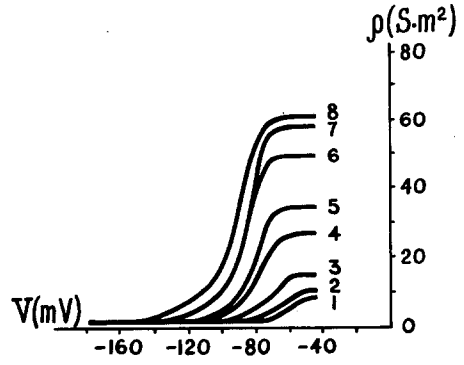


Fig. 13. D-channel conductance activation curves obtained from SVCC's at various pH values in 2.5 mM K_2SO_4 + 0.1 mM $CaSO_4$ solution. Curves 1 to 8 correspond to 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 pH, and were obtained using equation $g = I/(V - V_K)$

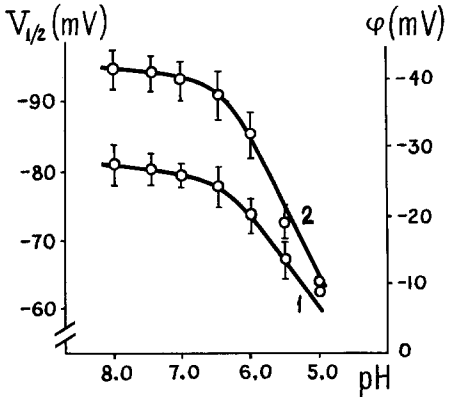


Fig. 14. D-channel activation curve shifts (changes of $V_{1/2}$) *vs.* pH of the medium. Dependences 1 and 2 have been obtained for 5 K^+ solution with 0.1 and 0.02 mM Ca^{2+}, respectively. The curves have been drawn as a result of calculations (details are in the text). The surface potential values are shown on the right-hand ordinate axis

Discussion

D- and H-Channel Transport and Activation Properties

Analysis of the IVCC's and the above considerations give good reasons for believing that the plasmalemma conductance is due to the two types of channels, here called D- and H-channels.

From the IVCC's it is possible to determine certain features of the potassium ion movement mechanism and, first of all, to establish whether the independence principle (Hodgkin & Huxley, 1952) is satisfied. If this is the case, the unidirectional ionic fluxes are proportional to the corresponding ion concentrations. For example, K^+ influx should increase proportionally to its external concentration. In our experiments, the inward current is determined by almost unidirectional K^+ influx at a sufficiently high hyperpolarizing voltage when the difference between the membrane voltage and the K^+ equilibrium potentials exceeds 150 mV. In this case, at 5 mM K^+ concentration in the external solution, the efflux is about 2% of the influx (if the independence principle is satisfied).

Table 1 presents potassium channel inward current values (I) taken from the IVCC's of Fig. 4 for different K^+ concentrations in the external solution. I_i is the value of current that would flow if the independence principle is satisfied. Therefore, I/I_i ratio shows deviation of the really flowing current from the value predicted on the basis of the independence principle.

From the data presented in Table 1 it follows that for D-channels the independence principle is satisfied. In H-channels the principle is not satisfied since in response to the potassium concentration increase the current rises to a smaller extent than predicted from the independence principle (*see* Table 1).

Using the tracer technique, Walker and Hope (1969) showed that the independence principle is violated for potassium ion flux through the plasmalemma of *Chara corallina* cells. In their experiments steady values of unidirectional fluxes were

measured in a wide range of voltages. Our experimental data show that the independence principle for the potassium ion flux in the membrane is satisfied only in the depolarization region when D-channels are activated, and is violated in the hyperpolarization region. Obviously, Walker and Hope obtained an integral estimate neglecting the potential dependence of the plasmalemma properties.

The fact that the independence principle is satisfied in D-channels strongly suggests that the ionic movement in the channels follows the free diffusion mechanism (Markin & Chizmadzhev, 1974). The latter is expressed by the "constant field" model (Goldman, 1943; Hodgkin & Katz, 1949). Taking into account that fixed negative surface charges concentrate the solution cations adjacent to the membrane, the equation for the current flowing through the membrane under the present conditions is

$$I = P_K \frac{F^2}{RT}\left(V - \frac{RT}{F}\ln\gamma\right)\frac{(C_K^o e^{VF/RT} - C^i)\gamma}{e^{VF/RT} - \gamma} \qquad (1)$$

where V is the potential difference on the membrane; V_S is the surface potential; C_K^o is K^+ concentration in the solution; $C^i = C_K^i + \alpha C_{Na}^i$; $\alpha = P_{Na}/P_K$; C_K^i, C_{Na}^i are the potassium and sodium concentrations close to the membrane inner side; P is the permeability coefficient; $\gamma = e^{V_S F/RT}$; F, R, T are used as conventionally in thermodynamics.

The IVCC shape may be described by Eq. (1). Figure 4*a* shows the experimental (points) and calculated (solid lines) dependencies. The latter were obtained using the following parameter values: C^i = 75 mM, $\gamma = 1.16$, $P_K = 6 \times 10^{-6}$ cm^{-1}. Good agreement of the predicted and experimental curves can be seen. The obtained values of C^i and P_K are in accord with the available reported data. The surface potential for $\gamma = 1.16$ is approximately -2 mV (a more detailed description of the plasmalemma surface potential is given below).

Agreement between the experimental IVCC's and curves calculated from Goldman's model confirms that ion movement in D-channels obeys the free diffusion mechanism, i.e. the assumption made above due to the fact that for D-channels the independence principle is satisfied. It is therefore clear that Goldman's equation should be used for interpretation of the experimental data only when D-channels are mostly activated, i.e. when the PD across the plasmalemma is not high. Hyperpolarization of the plasmalemma, for example, by diminishing the external K^+ concentration to 0.1 mM and lower can cause discrepancies between the model predictions and values observed. Surface charge changes due to various reasons should also be taken into account. Our experimental data suggest that a mechanism different from free diffusion should be used to account for the movement of potassium ions in H-channels.

Table 1. Comparison of inward currents (I) measured in D- and H-channels with currents (I_i) calculated on the basis of the independence principle

Plasmalemma voltage (mV)	Currents (I, A/m^2)	K^+ concentration in external solution (mM)		
		2.0	5.0	10.0
	D-channels			
−280	I	0.14	0.33	0.65
	I/I_i	1.0	0.94	0.93
−240	I	0.13	0.29	0.57
	I/I_i	1.0	0.91	0.89
	H-channels			
−280	I	0.11	0.19	0.30
	I/I_i	1.0	0.67	0.54
−240	I	0.08	0.14	0.21
	I/I_i	1.0	0.67	0.50

Both types of channels are potential dependent. Under normal conditions D-channels are activated at plasmalemma depolarization above −80 to −100 mV, and their activation accounts for virtually all the increase of the plasmalemma conductance at this voltage range. Activation of H-channels, clearly illustrated under special conditions, takes place at plasmalemma hyperpolarization beyond −80 to −100 mV. The activation of H-channels is low; the conductance and current are approximately doubled. As the CV diminishes, the membrane conductance and inward current amplitude decrease (Fig. 3, curves 4 and 5) due to inactivation of D-channels which remain open at −100 mV. This effect masks the activation of H-channels under normal conditions.

In the hyperpolarization range below −140 mV, H-channels are basically responsible for the plasmalemma conductance. It should be pointed out that the voltage dependence of K^+ conductance for *Nitella* and *Nitellopsis* cell membranes were reported by Kitasato (1973) and Krawczyk (1975). Nevertheless, evaluating membrane conductance at various plasmalemma voltages, the above authors postulated that the IVCC's are linear, while the results of our investigations demonstrate essential nonlinearity of the voltage-current curves (Fig. 3).

The identified two types of potassium channels on plasmalemma, which are activated at opposite changes of voltage across the membrane, account for certain contradictions in the experimental data. As is known, the plasmalemma permeability to K^+

ions, calculated from the experimental conductivity values in terms of Goldman's model, decrease in a single-component KCl solution with an increase in potassium concentration, and in some cases the permeability increases when the ionic strength remains constant ($C^o_K + C^o_{Na} = \text{const}$) (Yurin et al., 1977). The initial potential difference of the plasmalemma in the experiments under discussion was −140 to −150 mV. As was shown above, under these conditions H-channels are responsible for the plasmalemma conductance. As K^+ concentration in the solution rises, K^+ permeability diminishes since the independence principle in H-channels is violated and they are inactivated by depolarization. As the ionic strength in a single-component KCl solution increases (as a result of K^+ concentration increase) the activation curve of D-channels is shifted along the voltage axis towards depolarization due to a negative surface charge on plasmalemma. Therefore as K^+ concentration increases, D-channel activation may not occur in response to plasmalemma depolarization. In this case, as is shown above, the fall in the membrane permeability to K^+ is accounted for by H-channels. When the ionic strength of the solution is maintained constant the activation curve of D-channels is not shifted and they may be activated as the K^+ concentration increases. As a result K^+ permeability may rise.

Now the mechanism of "inductive" and "hyperpolarization" responses of *Nitella* cell plasmalemma in a solution containing a high concentration of K^+ (Kishimoto, 1966; Bradley & Williams, 1967) becomes evident. A sharp drop of conductance observed upon plasmalemma hyperpolarization results from inactivation of D-channels. It is also clear that D-channels are the potassium channels (Gaffey & Mullins, 1958; Haapanen & Skoglund, 1967; Oda, 1975) which, being activated upon plasmalemma depolarization during the rising phase of the action potential, determine its repolarization during the falling phase.

Thus the experimental data obtained prove that both types of channels are potential dependent and are activated upon shifting along the voltage axis in opposite directions. Slow inactivation has been found for D-channels. A qualitative similarity of the activation characteristics for D-channels and potassium channels of animal cell membranes can also be pointed out.

Selectivity

There are few publications on the selective properties of plant cell plasmalemma. Moreover, the ionic selectivities measured until now (Zubov, 1975) are averaged since two different populations of potassium channels existing on the plasmalemma at rest were not taken into account. On the other hand, the ionic selectivity of resting membranes was determined in terms of Goldman's theory which is valid for D-channels only.

The membrane ionic selectivity can be evaluated in terms of Goldman's theory using the equation (Lev, 1975; Hille, 1971, 1972):

$$V^o_j - V^o_K = \frac{RT}{F} \ln \frac{P_j C^o_j}{P_K C^o_K} \tag{2}$$

where V^o_j and V^o_K are zero-current potentials, C^o_j and C^o_K are the concentrations of ion under study and potassium, respectively, in an external solution.

The ionic selectivity of channels may be determined from the ratio of currents (by IVCC's) at the appropriate substitution of cations. If the independence principle is satisfied both estimates should be identical (Table 2).

D-channel selectivity series (in the order of permeability decrease) may be presented as follows: $K^+ > Rb^+ > NH_4^+ > Na^+ \geq Li^+ > Cs^+ > TEA^+ \approx$ choline$^+$. The permeability values estimated from Eq. (2) and from the current ratio are not similar for all ions (Table 2). This may imply that the independence principle is violated for certain cations (NH_4^+, Rb^+ and others). For such ions as Cs^+ and TEA^+ discord of the values may be ascribed to the blocking effect.

On the other hand, it may be assumed that NH_4^+ cation passes through plasmalemma by the "amine uniport" mechanism (Walker, Beilby & Smith, 1979), rather than the potassium channels. However, experimental data obtained at our laboratory and partially published (Kudriashov & Goncharik, 1980) show that the "amine uniport" mechanism is activated in the cells only if they are specially pretreated. In our experiments dealing with K^+ channels, the cells were not subjected to such pretreatment. Therefore, NH_4^+ cations perhaps passed through potassium channels.

The difference between the two permeability evaluations for H-channels is much greater than for D-channels (Table 2). It can well be expected because the independence principle in H-channels is not satisfied even for K^+ ions. In this case, the current ratio gives a more appropriate estimation. The selectivity series for H-channels is similar to that of D-channels. This can be explained by the fact that both types of channels are K^+ selective, and the selectivity mechanism may be expected to be analogous in both types.

Comparison of cation crystalline radii and their relative permeabilities allows us to suggest that the selective filters of D- and H-channels are structur-

Table 2. K^+ channel selectivity characteristics

Cation	D-channels			H-channels		
	V_i (mV)	P_j/P_K	I_j/I_K ($V = -250$ mV)	V_i^o (mV)	P_j/P_K	I_j/I_K ($V = -210$ mV)
K^+	− 80	1.00	1.00	− 75	1.00	1.00
Rb^+	− 87	0.73	0.57	− 85	0.68	0.46
NH_4^+	−107	0.37	0.53	− 95	0.45	0.56
Na^+	−117	0.23	0.20	− 90	0.55	0.26
Li^+	−118	0.22	0.34	− 90	0.55	0.26
Cs^+	−127	0.16	0.11	−103	0.34	0.18
TEA^+	−140	0.10	0.07	−122	0.16	0.12
$Choline^+$	−140	0.10	0.14	−122	0.16	0.14

ally similar to the potassium channel filter in animal cell membranes. It is probably a pore, 3 Å in diameter, formed by oxygen atoms (Hille, 1973).

Blocking

Cs^+ block is potential dependent: for both types of channels only inward current was inhibited and blocking began at the zero-current potential. When the zero-current potential changed due to a decrease in potassium concentration in the external solution, onset of the blocking effect shifted to a new value of the potassium equilibrium potential for both types of channels. The block seems to depend on the direction of total current flowing through the channel, i.e. Cs^+ ions are knocked out by potassium ions leaving the cytoplasm. This is also confirmed by an increase of D-channel outward current in the presence of Cs^+ in the solution (Fig. 7). Low permeability of the plasmalemma to Cs^+ (*see* Table 2) implies that the block observed is associated with Cs^+ effect on the external side of the plasmalemma.

The data were quantitatively interpreted in terms of the potential dependent ion block model (Woodhull, 1973), according to which the ratio of the number of blocked channels to the total number of channels, equal to the current ratio with and without a blocker, may be expressed as follows:

$$I_{K+Cs}/I_K = [1 + (C_{Cs}/K) \exp(-\delta VF/RT)]^{-1} \quad (3)$$

where K is the dissociation constant of the ion-binding site complex at $V = 0$; V is the membrane voltage; C_{Cs} is Cs^+ concentration in the solution; δ is the slope of the blocking potential dependence (the dependence of I_{K+Cs}/I_K on the membrane voltage), and within the model it expresses the part of the membrane potential which drops over the channel portion the ion passes before it reaches the binding site and induces blocking (Adelman & French, 1978). In accordance with the constant field approximation (Markin & Chizmadzhev, 1974), δ expresses the relative distance from the binding site to the membrane surface.

Figure 9*a* shows the complex character of D-channel Cs^+ block. The curves drawn according to Eq. (3) using the appropriate parameters (*see* the legend to Fig. 9*a*) fit the experimental points near the reversal potential. There is no fitting in the hyperpolarization range. In general the current-dependent block data (Seyama et al., 1980) agree with our experimental data less since the slope of I_{K+Cs}/I_K potential dependence for the current-dependent block is considerably higher than our experiment shows (Fig. 9*a*).

Cs^+ concentrations about 1 mM cause δ and K to increase from 0.9 to 2.4 and from 40 to 1800 mM, respectively, thus suggesting that the block character has changed. It is possible that Cs^+ increase causes the mono-ion block to change to a bi-ion block. Calculations performed (Hille & Schwartz, 1978) show that δ may equal three in a three- or four-site channel. Cs^+ block potential dependence for H-channels (Fig. 9*b*) fits Eq. (3). The channel is blocked by one ion, $\delta \approx 0.5$, $K \approx 50$ to 100 mM.

The potential dependence of the TEA^+ blocking effect on H-channels is similar to Cs^+ effect (only inward current was inhibited), while in D-channels TEA^+ inhibits both the inward and outward currents. From the above results it follows that the main blocking effect within the first 10 to 15 min after the blocker has been added to the solution is associated with its adsorption on the external part of the channel. It seems that, after the cell has been kept in TEA^+ solution for 2 hr, TEA^+ enters the cytoplasm and blocks D-channels on the inside. Therefore, only the results obtained during the first 10 to 15 min after TEA^+ addition to the solution are discussed here.

On the basis of the dose-effect dependence for D-channel blocking it has been found that one ion is bound with each channel, and K is 15 mM. Since the block is potential independent this may imply that D-channel mouth is not wide enough for a TEA^+ ion (8.5 Å dia.) to pass the channel portion on which a considerable part of the membrane potential drops. TEA^+ ion seems to be bound so strongly that the outward potassium current cannot effectively unblock the D-channel (there is no knock-out effect). Quantitative interpretation of the data in terms of the above potential dependent block model (Eq. 3, Fig. 9*c*) gives the following parameters for H-channels: $\delta = 0.24$, $K \approx 140$ to 240 mM, one channel is blocked by one TEA^+ ion.

As a result of studying the D-channel H^+ block we found the pK of H^+ binding sites to be equal to 7.1. The value rules out the possibility of effects associated with the action of fixed charges in the cell wall (pK of the corresponding groups is less than 5 (Ivanov, 1971)) and the plasmalemma surface charges (pK 5.8, our data in this paper) on the concentration of cations near the membrane. H^+ block potential independence in terms of the model used here, together with the data on constant pH value in *Nitella* cell cytoplasm at varying H^+ concentration in the external solution (Bobrov et al., 1980) suggests that the H^+ binding sites are located close to the external surface of the plasmalemma.

Thus interpretation of the experimental results in terms of the model used allows to suggest a substantial structure difference between D- and H-channels. D-channel inlet from the side of the external solution can contain several Cs^+ ions at a time. The channel mouth is not wide enough to pass a TEA^+ cation and has an H^+ binding site close to the membrane surface. H-channel mouth from the side of the external solution is somewhat wider (a TEA^+ ion passes 0.24 of the plasmalemma thickness), can contain one Cs^+ ion and does not have any H^+ binding sites.

Plasmalemma Surface Charges and Their Effects on K^+-Channel Transport Properties

Previous evaluations (Sokolik, 1982) made in terms of Gouey-Chapman's theory of an electric double layer (Lakshminarayanaiah, 1977) show that on varying the concentration of Ca^{2+} ions in the range under study their adsorption on fixed charges is essential since the screening effect in a 5 mM K^+ solution becomes noticeable beginning with 0.3 mM Ca^{2+} concentration. Therefore, it may be assumed that the surface potential is changed due to partial compensation of fixed charges caused by competitive Ca^{2+} and H^+ adsorption; and in this case a calcium ion may occupy one or two binding sites (Mozhaeva & Naumov, 1972). The best agreement of the predicted and experimental dependences (Fig. 14) is observed for a two-site Ca^{2+} binding and the following parameters: fixed-charge surface density 1/800 to 1/1200 $Å^{-2}$; pK_H 5.75 to 5.80, pK_{Ca} of 2.9 to 3.1. The fixed charge surface density obtained in our experiments is lower than the available data for animal cell membranes. The differences between H^+ and Ca^{2+} binding constant are still greater (Begenisich, 1975; Drouin, 1976; Carbone et al., 1978). This suggests that the nature of groups carrying negative fixed charges on the *Nitella* cell plasmalemma is different from similar groups on the animal cell membranes.

The negative surface charge field concentrates cations in the layer adjacent to the membrane in accordance with Boltzman's law. In view of the fact that the independence principle is satisfied for K^+ movement in D-channels, the logarithm of conductance versus the surface potential should be close to linear with a 58-mV slope. When Ca^{2+} and H^+ concentrations are varied, the dependence of this form is observed for Ca^{2+} over the entire range and for H^+ at pH 5.0 to 6.5. At pH above 6.5 the surface potential remains almost constant (Fig. 14) while conductance keeps rising as H^+ concentration decreases (Fig. 13). This effect cannot be attributed to fixed anions of the cell wall, because, as was mentioned above, pK_H of the respective groups is below 5. It is clear that the observed conductance rise results from D-channel deblocking due to H^+ desorption from the binding sites as the solution pH increases.

In conclusion it should be pointed out that absence of Ca^{2+} and H^+ effect on H-channel conductance, taking into account existence of fixed surface charges close to D-channel mouths implies that the mouths of the majority of D- and H-channels on the plasmalemma are considerably spaced apart as compared with the thickness of the electric double layer. Surface charges localized near D-channel mouths make it possible to change the plasmalemma conductance from D- to H-channels and back; for example, a decrease in Ca^+ and H^+ concentration in the medium increases the surface potential and diminishes the absolute voltage value across the membrane to cause D-channel activation. This probably explains why Goldman's equations give a better description of the electrophysiological characteristics of the characean algae plasmalemma in calcium-free solutions (Yurin et al., 1977).

Thus, besides a number of common properties,

there are substantial differences between the two types of channels. The fact that Cs^+, TEA^+ and H^+ block D- and H-channels in a different manner suggests that the channels have entrance parts of different structure. The experiments with Ca^{2+} and H^+ indicate that the entrance parts of H-channels are situated outside the electric double layer produced by fixed negative charges located near D-channels, i.e. the two types of potassium channels are spaced apart on the plasmalemma and are constituted by different molecular structures.

Cation transfer across the resting plasmalemma, as across an excitable membrane, is a two-stage process. The first stage is the ion transfer reaction itself, whose characteristics can be traced from the IVCC's analysis. In our case, the process is represented by a nonlinear function of the membrane potential. The IVCC nonlinearities are different for both types of channels.

The second key factor is the gating mechanism controlled by the electric field in the membrane. It should be pointed out that these properties are different for both types of channels; for example, the potential dependence of the channels is opposite in sign.

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