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Are Redox Reactions Involved in Regulation of K^+ Channels in the Plasma Membrane of *Limnobium stoloniferum* Root Hairs?[1]

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The effects of the impermeant electron acceptor hexacyanoferrate III (HCF III) and the potassium channel blocker tetraethylammonium (TEA) on the current-voltage relationship and electrical potential across the plasma membrane of *Limnobium stoloniferum* root hairs was investigated using a modified sucrose gap technique. One millimolar HCF III immediately and reversibly depolarized the membrane by 27 mV, whereas the effect on the trans-membrane current was markedly delayed. After 6 min of treatment with this electron acceptor, outwardly rectifying current was inhibited by 50%, whereas the inwardly rectifying current was activated approximately 3-fold. Ten millimolar TEA blocked both outward (65%) and inward (52%) currents. Differential TEA-sensitive current was shown to be blocked (55%) by HCF III at −20 mV and was shown to be stimulated (230%) by this electron acceptor at −200 mV. The inward current at −200 mV was eliminated in the absence of K^+ or after addition of 10 mM Cs^+ and was not affected by addition of either 10 mM Na^+ or Li^+, independent of the presence of HCF III. The addition of any alkali cation to the external medium decreased the outward current both in the presence and in the absence of HCF III. The membrane depolarization evoked by HCF III did not correlate with the corresponding modification of the inward current. HCF III is proposed to activate inwardly rectifying potassium channels and to inactivate outwardly rectifying potassium channels. It is concluded that the plasma membrane depolarization did not result from modulation of the potassium channels by HCF III and may originate from trans-plasma membrane electron transfer.

Plant cells exhibit a constitutive activity that reduces external impermeable artificial electron acceptors like HCF III (Novak and Ivankina, 1978; Craig and Crane, 1981; Böttger and Hilgendorf, 1988). This activity has been ascribed to a ubiquitous "standard" redox system operating in the plant plasma membrane. The physiological role of the system is still unclear (Bienfait and Lüttge, 1988; Böttger et al., 1991).

Accessing the redox system with artificial electron acceptors leads to an immediate increase in net proton secretion (Federico and Giartosio, 1983; Böttger and Lüthen, 1986), an increase (Grabov et al., 1993) or decrease (Ullrich and Guern, 1990) in cytoplasmic pH, and depolarization of the plasma membrane (Ivankina and Novak, 1988; Döring et al., 1990; Ullrich and Guern, 1990; Grabov et al., 1993). The nature of the link between redox activity and proton excretion is still under debate (Novak and Ivankina, 1978; Böttger and Lüthen, 1986; Rubinstein and Stern, 1986; Ivankina and Novak, 1988, Döring et al., 1990).

HCF III also inhibits the net K^+ influx in the majority of plant species investigated (Kochian and Lucas, 1985; Rubinstein and Stern, 1986; Ivankina and Novak, 1988). Because potassium transport across the plasmalemma is tightly coupled to trans-membrane proton motive force (Kochian and Lucas, 1988), such an inhibition may be accounted for by redox-evoked perturbations of E_m and is in accord both with hypotheses concerning a direct link between electron and proton transport (Ivankina and Novak, 1988) and those concerning an indirect link (Rubinstein and Stern, 1986).

Apparently, accessing the plasmalemma electron transfer system with redox agents may also trigger some regulation processes apart from modulation of an energy-transducing system. Thus, inhibition of K^+ influx in corn root segments by exogenous NADH was shown by Kochian and Lucas (1985) to be a result of a cascade of regulatory events similar to wound response, suggesting participation of the plasmalemma redox system in the regulation of K^+ translocation (Kochian and Lucas, 1991). Because K^+ channels may represent a pathway for potassium transport (Kochian and Lucas, 1988; Schroeder, 1988; Grabov, 1990; Blatt, 1991), they may be a target for such regulation. Indeed, a number of data indicate that K^+ channels of yeast, plant, and mammalian cells are susceptible to such redox reagents as active oxygen species (Kuo et al., 1993) and sulfhydryl-group reactants (Bertl and Slayman, 1990; Ruppersberg et al., 1991; Thiel, 1991; Spalding et al., 1992).

Cell depolarization after addition of HCF III is proposed to be a result of modulation or induction of the trans-plasma membrane electron transfer (Ivankina and Novak, 1988;

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Abbreviations: E_m, trans-plasma membrane electrical potential; E_K, K^+ equilibrium electrical potential; G, slope conductance; G/V, slope conductance/voltage; HCF II, hexacyanoferrate II (ferrocyanide); HCF III, hexacyanoferrate III (ferricyanide); I, membrane current; I_F, membrane current in the presence of HCF III; I_O, membrane current in control medium; I_T, membrane current in the presence of TEA; I_{TF}, membrane current in the presence of both TEA and HCF III; I/V, current/voltage; K_D, dissociation constant; i, total membrane current; TEA, tetraethylammonium.

Döring et al., 1990; Ullrich and Guern, 1990; Grabov et al., 1993). However, a modification of the K^+ channels may also be responsible for this process since they are tightly involved in cell electrogenesis.

The aim of the present investigation was to study the impact of HCF III on the potassium channels and its role in the change in E_m. We used root hairs for our investigation because they have a plasma membrane with an extremely high potency for ion uptake. The modified Suc-gap technique developed recently (Grabov, 1990) provided application of voltage clamp on the delimited part of root hair plasmalemma.

MATERIALS AND METHODS

Plant Material

Limnobium stoloniferum (G. F. Mey) Griseb. (Syn. *Trianea bogotensis* Karst.) (family Hydrocharitaceae), a water plant with large root hair cells, was grown swimming under a light/dark regime of 16/8 h at 25 μmol photons s^{-1} m^{-2} nutrient on medium consisting of a 8.3% Hoagland solution with addition of 0.2 mg/L $CuSO_4$, 3 mg/L H_3BO_3, 1.4 mg/L $MnSO_4$, 0.8 mg/L $ZnSO_4 \cdot 7\ H_2O$, 0.2 mg/L Na_2MoO_4, and 9.5 mg/L Fe^{2+} as EDTA complex.

Roots about 50 mm in length were excised just before being placed into the experimental chamber. Measurements were performed on root hairs 1.4 to 2.0 mm in length located at a distance 10 to 20 mm from the root tip.

Experimental Chamber

The experimental chamber (Fig. 1) consisted of two flow-through compartments separated by a divider 0.6 mm thick. A hole of 70 to 75 μm in diameter in the divider connected both compartments. The diameter of the hole was close to the diameter of the root hair (55–60 μm). The root was placed into the left (root) compartment, and one root hair was pushed carefully through the hole under microscopic control. After this procedure the tip of the root hair was exposed to the right (experimental) compartment while the whole root and base of the root hair remained in the left one.

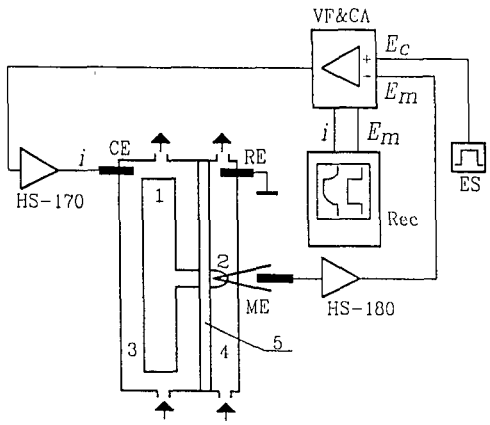

Figure 1. Set-up for space-clamp of a root hair. 1, Root; 2, apical part of root hair under study; 3, compartment with Suc solution (3.2 mM Suc + 10 μM $CaCl_2$); 4, compartment with experimental medium; 5, divider. ME, Microelectrode; CE, current electrode; RE, reference electrode; HS-180 and HS-170, head stages; VF&CA, voltage clamp unit consisting of a VF 102 microelectrode amplifier and a CA 100 voltage clamp amplifier; ES, electrostimulator; Rec, recorder; arrows, inlets and outlets (by suction) for solutions.

The root compartment was continuously rinsed (3 mL min^{-1}) with a solution of low conductivity (11 μS cm^{-1}). This solution was isoosmotic to the growth medium and consisted of 3.2 mM Suc and 10 μM $CaCl_2$. The experimental medium bathed the tip of root hair at the rate of 1 mL min^{-1}. The composition of the experimental medium varied, depending on the aim of the experiment.

The bath surface of the Suc solution was kept precisely at 4 mm above that of the experimental medium. This provided a small but continuous flow of Suc solution through the gap around the root hair in the hole, thus avoiding any possible leakage of electrolytes from the experimental compartment into the gap. Consequently, a large gap resistance of more than 200 MΩ was obtained independently of the composition of the experimental medium.

Electrical Configuration

The voltage-clamp unit (Fig. 1) consisted of a VF-102 dual microelectrode amplifier with HS-170 and HS-180 headstages and a CA-100 voltage-clamp amplifier (Biologic, Echirolles, France).

The E_m was measured by a microelectrode filled with 0.5 M KCl and was led through an Ag/AgCl half-cell to an HS-180 preamplifier mounted on the micromanipulator. E_m and the command voltage from a home-made electrostimulator were compared by a voltage-clamp unit. The resulting difference voltage signal was converted by the HS-170 into the current passed through the current electrode across the plasma membrane. The HS-170 was mounted on the experimental chamber. The current electrode was placed in the root compartment and consisted of an Ag/AgCl half-cell and a glass capillary filled with 0.5 M KCl. The reference electrode placed in the experimental compartment had the same design. The membrane voltage and current were recorded by a Servogor 460 two-channel pen recorder (Goerz, Wien, Austria).

In this configuration the experimental medium was zeroed by the reference electrode and was insulated by the gap from the solution bathing the base of the root hair. Therefore, the microelectrode registered only the voltage across that part of the plasma membrane that was rinsed by the experimental medium. In voltage-clamp mode the electrical current between current and reference electrodes was supplied by a voltage-clamp amplifier. Because of the high resistance of the gap, the transfer of this current between two compartments of the chamber was mediated by the root symplast. Thus, the current crossed the plasma membrane twice: once in the root compartment and once in the experimental compartment. The trans-plasma membrane current in the experimental compartment resulted in the change of E_m recorded by the microelectrode. The current across the plasma membrane in the root compartment did not influence the voltage measured by the microelectrode. In this way we were able to clamp the

voltage of a strongly delimited part of the root hair plasmalemma. This configuration represents a modification of the Suc-gap technique (Coburn et al., 1975).

Experimental Protocol

Prior to impalement the tip of the root hair was rinsed for 45 to 60 min with a control medium. Measurements were performed about 30 min after impalement, when the membrane potential had stabilized. The location of the microelectrode tip in the cytoplasm of the root hair has been recently discussed (Grabov, 1990; Ullrich and Novacky, 1990). During the measurements of the membrane currents, the set-up was temporarily switched to voltage-clamp mode with a baseline potential of −100 mV. In this mode a bipolar staircase of voltage-clamp commands with 20-s pulses to various potentials separated by 25 s at the baseline clamp potential was applied (Fig. 2A). After application of the voltage scan the free-running membrane potential recovered completely, suggesting that the cell was not injured by this procedure. Tail currents also disappeared during the time between pulses. The membrane currents were stable within 20-s pulses at clamp voltages in the range of −200 to −20 mV. However, the current "ran down" in some experiments at voltages stepped to 0 mV, as depicted in Figure 2B. Only the stable current records were used for plotting *I/V* relationships. Data from one cell are usually presented; however, experiments were repeated three or more times and the results were at least qualitatively similar. The typical protocol is represented by a condensed record from a single cell in Figure 2, A and B.

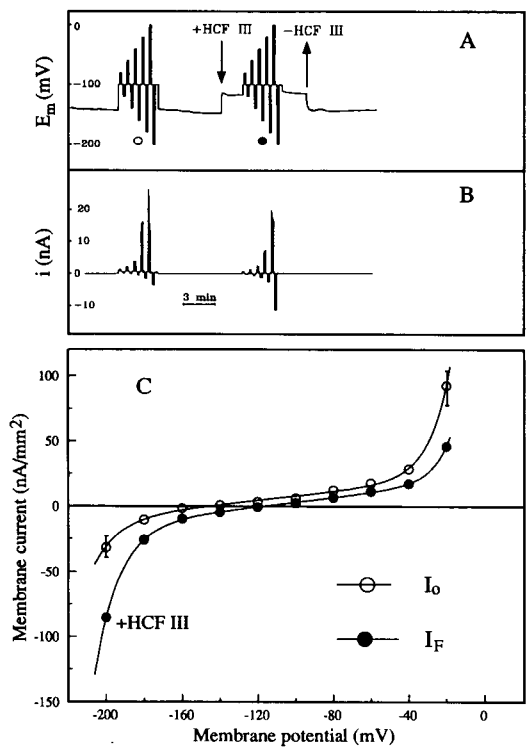

Figure 2. E_m (A), *i* (B), and *I/V* relationship (C) in the absence (O) and in the presence (●) of 1 mM HCF III. Control medium contains 10 mM KCl and 10 mM $CaCl_2$. KCl was reduced to 7 mM in the presence of HCF III to avoid a change in potassium concentration. Symbols marking voltage scans in A and *I/V* relationships in C are cross-referenced. The original traces are depicted in A and B, and the data points in C represent the mean of four and two measurements in the absence and in the presence of HCF III, respectively, performed with a single cell. SE values were calculated for the control curve only and are indicated as vertical bars. The error bars are not shown if they were smaller than the symbols. The lines represent the least square fit of the sum of two exponential and one linear function to the experimental data.

Chemicals

A 1-M stock solution of TEA was prepared overnight and kept at +4°C. Sodium ferricyanide was obtained from ICN Pharmaceuticals (Costa Mesa, CA). Other chemicals were purchased from Merck (Darmstadt, Germany).

RESULTS

Effect of HCF III on E_m and the *I/V* Curve

As is seen in Figure 2A, the E_m of the root hair tip reached −145 mV in the control medium containing 10 mM KCl and 10 mM $CaCl_2$, which is roughly 80 mV more negative than the E_K calculated from data by Zlotnikova and Vakhmistrov (1982). Addition of 1 mM HCF III to the medium resulted in an immediate and reversible depolarization of the plasma membrane to new steady-state level at −118 mV (Fig. 2A). HCF III was simultaneously reduced by *L. stoloniferum* root cells at a rate of 1.5 nmol s^{-1} g^{-1} fresh weight as was shown earlier (Grabov et al., 1993).

In control medium the *I/V* relationship was linear in the voltage range between −160 and −60 mV (Fig. 2C). Outward and inward rectifying currents were observed at more positive and negative clamp voltages. The sum of two exponential and one linear function fitted well to the dependency of membrane current on membrane voltage (Fig. 2C). The parameters of linear and nonlinear components were obtained by the least square procedure. The differentiation of the *I/V* relationship with the use of these parameters enabled us to obtain the value for membrane *G* (Fig. 3). *G* was clearly voltage dependent and drastically increased at $E_m < -160$ mV and $E_m > -60$ mV.

Membrane currents were scanned within 2 to 6 min after application of 1 mM HCF III (Fig. 2, A and B). The typical *I/V* profile consisting of two rectifying limbs was also maintained after this treatment. However, the inward current at −200 mV was increased by a factor of 3, but the outward current was inhibited by 50% by HCF III. The reversal potential was shifted by 27 mV toward more positive values with respect to the control *I/V* curve (Fig. 2, B and C). Application of the reduced form of HCF II had no impact on membrane potential or the *I/V* relationship (data not shown).

The *G* of the plasma membrane in the presence of HCF III was increased by 3.9 S/m^2 (220%) at −200 mV, it was reduced by 3.5 S/m^2 (53%) at −20 mV, and it remained practically invariable in the range of membrane voltage of −130 to −70 mV (Fig. 3). *G* measured at reversal potentials in the absence and in the presence of HCF III also did not differ

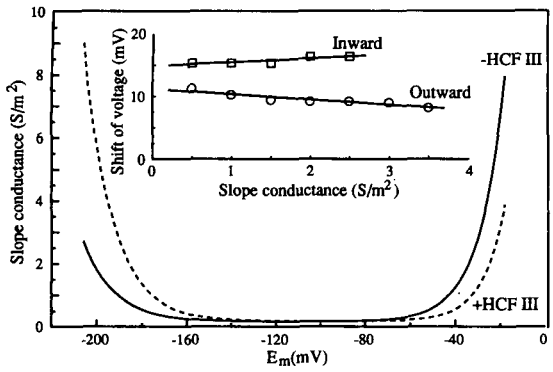

Figure 3. G of the plasma membrane before (solid line) and after (dashed line) application of 1 mM HCF III, obtained as derivatives of the I/V curves in Figure 2. The shift in the membrane potentials resulting in the same conductance before and after addition of HCF III is shown in the inset for the outward and the inward rectifier (O and □, respectively).

(0.19 and 0.18 S/m^2, respectively). In principle, the action of HCF III on membrane conductance can be described as an approximately parallel shift of the rectifying limbs of the G/V curve toward the more positive voltages (Fig. 3) by 15 to 16 mV and 8 to 11 mV for the inward and outward rectifier, respectively (Fig. 3, inset).

Effect of TEA on E_m and the I/V Curve in the Absence and in the Presence of HCF III

TEA, a well-known blocker of K^+ channels (Tester, 1988), transiently depolarized the plasma membrane by 11 mV with subsequent permanent depolarization of about 6 mV (Fig. 4A). Fifty-two percent of inward current at clamped potential −200 mV and 65% of outward current at −20 mV were blocked by TEA (Fig. 4B, I_T).

The blocking effect of TEA on plasma membrane G (Fig. 5) was clearly pronounced at a rectification range of membrane voltage (-160 mV $> E_m > -60$ mV). The extreme (83%) blockade of conductance was observed at −20 mV. At voltages near E_K (−60 to −80 mV), the blocking effect of TEA was practically not detectable (Fig. 5, inset).

In the presence of 10 mM TEA, application of 1 mM HCF III depolarized the cell with the same time course and to approximately the same E_m (−122 mV) as in the absence of TEA (Fig. 4A). HCF III stimulated inwardly rectifying current even in the presence of TEA but to a lesser extent (Fig. 4B). The outward current at −20 mV was further inhibited by 45% after addition of 1 mM HCF III to the medium containing 10 mM TEA. Generally, both rectifying currents were less sensitive to HCF III in the presence of TEA.

The TEA-sensitive currents were estimated by subtraction of the current obtained in the presence of TEA (Fig. 4B, I_T) from the control (Fig. 4B, I_O) and are shown in Figure 6A (I_O-I_T). The HCF III-dependent currents are demonstrated in Figure 6B (□) as the difference of currents obtained in the absence (I_O, Fig. 2C) and in the presence (I_F, Fig. 2C) of HCF III. The profile of the differential current (I_O-I_F) clearly demonstrates that primarily the rectifying currents out of the

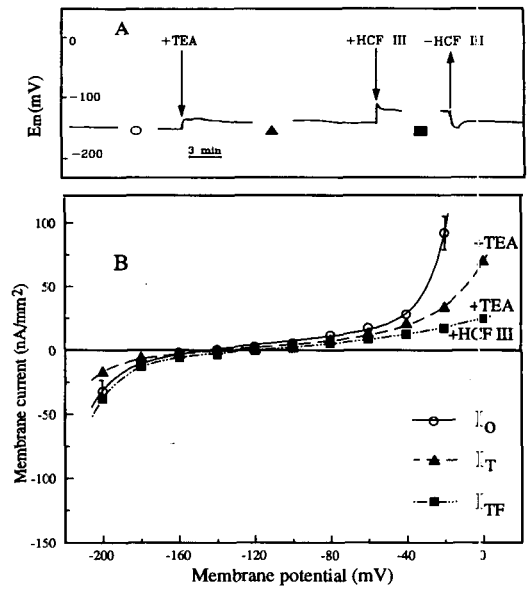

Figure 4. E_m (A) and I/V relationships before (O) and after successive application of 10 mM TEA (▲) and 1 mM HCF III (■) to the control medium containing 10 mM KCl and 10 mM $CaCl_2$. KCl was reduced to 7 mM in the presence of HCF III to avoid the change of potassium concentration. The application of voltage scans (omitted) in A is marked by symbols cross-referenced with the I/V relationships (B). The original traces are depicted in A, and the data points in B represent the mean of four measurements without addition (control) and two measurements for each consequent addition. SE values were calculated for the control curve only and are indicated as vertical bars. The error bars are not shown if they were smaller than the symbols. The lines represent the least square fit of the sum of two exponential and one linear function to the data.

range of I/V linearity (−160 to −60 mV) were affected by HCF III.

Using I/V curves obtained in the presence of both TEA and HCF III (I_{TF}, Fig. 4B), additional differential I/V relationships were plotted in Figure 6 (I_T-I_{TF} and I_F-I_{TF}). The differential curve I_T-I_{TF} represents the HCF III-dependent current

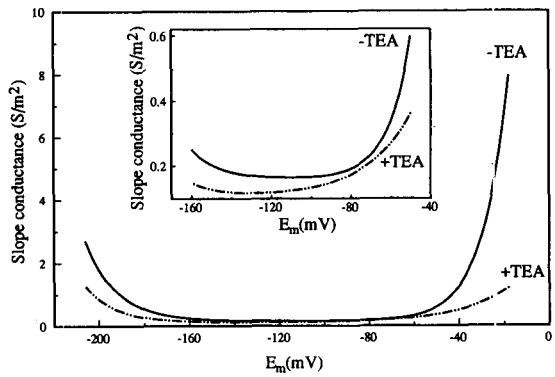

Figure 5. G of the plasma membrane before (solid line) and after (dashed line) application of 10 mM TEA, obtained as derivatives of the I/V curves in Figure 4. The same curves were shown in more detail in the inset for E_m of −160 to −50 mV.

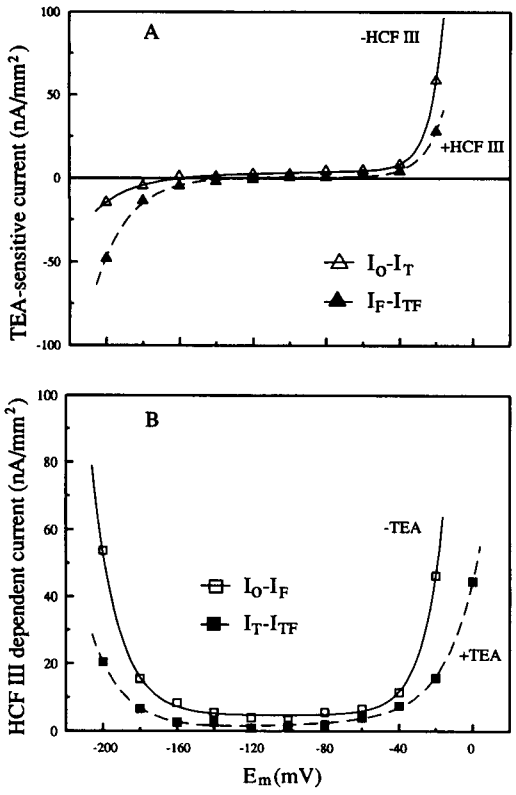

Figure 6. TEA-sensitive current (A) in the absence (△) and in the presence (▲) of 1 mM HCF III, and HCF III-dependent current (B) in the absence (□) and in the presence (■) of 10 mM TEA. Currents were calculated from data in Figures 2 and 4 as indicated in the panels.

in the presence of TEA (Fig. 6B). As shown in Figure 6B, more than 60% of HCF III-dependent current was blocked by TEA. The largest blockade was observed for rectifying currents (34 nA/mm² at −200 mV and 31 nA/mm² at −20 mV) with respect to linear current (2 nA/mm² at −100 mV). The differential curve I_F-I_{TF} in Figure 6A represents the modulation of the TEA-sensitive current by 1 mM HCF III. The strongest effects were again observed at the voltage extremes: 50% inhibition at −20 mV and 230% stimulation at −200 mV.

Kinetics of the HCF III Effect on E_m and I over a Long Time Scale

In further experiments (Fig. 7) the kinetics of E_m and I were investigated over a long time scale to distinguish direct effects of HCF III from regulatory events or putative secondary changes in cell metabolism. The currents measured at clamped voltages of −200 and −20 mV represent the activities of the inward and outward rectifiers, respectively.

The plasma membrane in these experiments was periodically clamped at a voltage of −100 mV that was subsequently stepped to −20 and −200 mV. Free-running E_m was sampled during the intervals between clamping. The composition of the medium was also periodically changed. Such an approach enabled us to perform quasi-simultaneous recording of free-running E_m and I clamped at different voltages in different media on a single cell.

Although depolarization of the plasma membrane occurred immediately after HCF III application, the activation of the inward current was time dependent and approached the steady state only within about 90 min after this treatment (Fig. 7, open symbols). The inhibition of outward current was faster and occurred within less than 10 min. The currents at a voltage of −100 mV were close to zero throughout the experiment and were practically unaffected by HCF III (data not shown).

Influence of External Ionic Composition and HCF III on E_m and I

Replacement of K^+ with Na^+ in the bath resulted in hyperpolarization by 30 and 12 mV in the absence and in the presence of 1 mM HCF III, respectively (Fig. 7A). As a consequence the depolarization evoked by HCF III was more pronounced by 18 mV in K^+-free medium.

The distinction between outward currents obtained in K^+-free and K^+-supplied baths was not clearly revealed in the absence of HCF III. The inhibition of outward current by HCF III in K^+-free medium was delayed, approaching steady state in about 1.5 h, and this current remained larger with respect to the control (Fig. 7B). The inward current was abolished in K^+-free medium even in the presence of

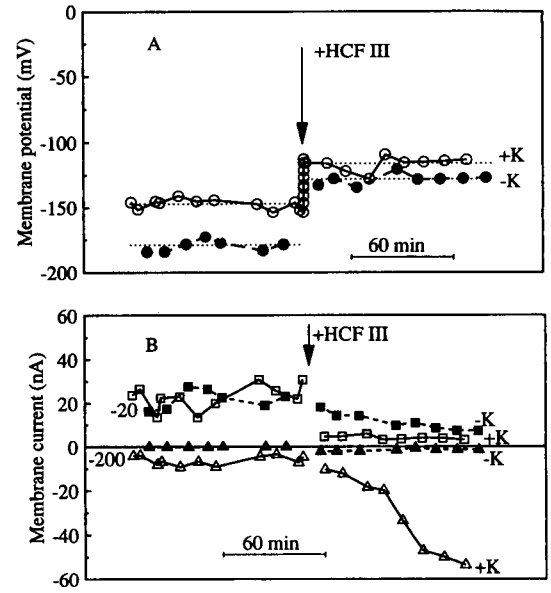

Figure 7. Impact of 1 mM HCF III on trans-plasmalemma resting potential (A) and current (B) in presence (open symbols) and absence (filled symbols) of K^+ in the medium. Dotted lines in A represent average E_m at each treatment. Experimental conditions were as in Figure 2. Sodium chloride and sodium ferricyanide were applied instead of the corresponding potassium salts in a K^+-free solution. Clamped potential of −20 (squares) and −200 (triangles) mV is indicated for the current curves. All parameters were recorded on the same cell by temporal switching of the bath (K^+ or Na^+), voltage clamping (on or off), and stepping the voltage clamp (to −20 or −200 mV from a holding potential of −100 mV).

HCF III. However, such a treatment stimulated inward current by about 600% in K^+-supplied medium. The currents clamped at −100 mV were small and were little affected by K^+, Na^+, or HCF III in the bath (data not shown).

Unfortunately, it was not possible to perform similar experiments with another alkali cation, since we had access only to the potassium and sodium salts of HCF III. Therefore, in the next set of experiments 1.5 mM potassium was always present in the base medium as 1.5 mM KCl or as 0.5 mM $K_3[Fe(CN)_6]$. Ten millimolar KCl, NaCl, LiCl, or CsCl was added to the basal medium as indicated in Figures 8 and 9.

The addition of K^+ depolarized the membrane by 20 mV in the absence of HCF III. Na and Li practically did not affect E_m, whereas Cs^+ hyperpolarized the cell by 22 mV (Fig. 8). The shift in E_m after the addition of cations was not dependent on HCF III (data not shown).

Addition of any cation to the bath decreased outward rectifying current, but maximal inhibition was observed in the presence of KCl and was measured at −20 mV (Fig. 9A). Linear current measured at −100 mV was not affected by this treatment (data not shown). Inward rectifying current was stimulated 4-fold upon addition of 10 mM KCl, whereas 10 mM CsCl completely blocked this current. No effect on the inward rectifier was observed with application of either NaCl or LiCl (Fig. 9A). Qualitatively the same results were obtained in the presence of 0.5 mM HCF III (Fig. 9B).

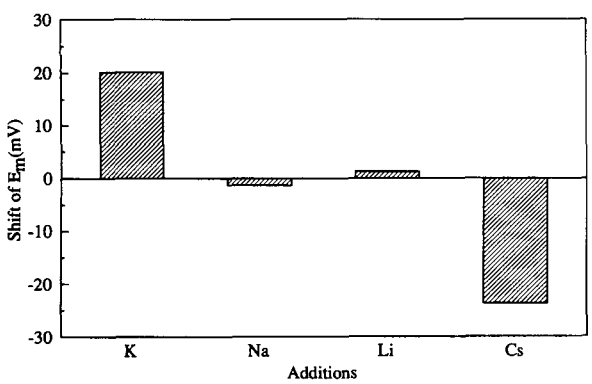

Figure 8. The change in E_m after the addition of 10 mM KCl, NaCl, LiCl, or CsCl to the medium containing 1.5 mM KCl and 10 mM $CaCl_2$.

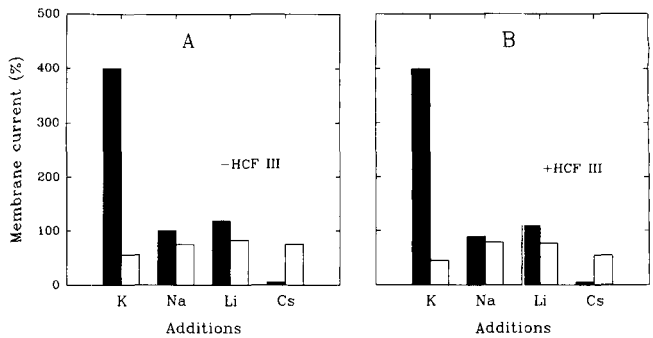

Figure 9. Effect of addition of 10 mM KCl, NaCl, LiCl, or CsCl on the trans-plasma membrane currents (% of control without additions) at −20 mV (open bars) and at −200 mV (filled bars) in the absence (A) and in the presence (B) of 0.5 mM HCF III. Before addition of cations, the medium contained 10 mM $CaCl_2$ and 1.5 mM KCl (A) or 0.5 mM $K_3[Fe(CN)_6]$ (B). The currents before addition of 10 mM alkali cation are assumed to be 100%.

DISCUSSION

The Origin and General Characteristics of Rectifying Currents

Analysis of tail and steady-state *I/V* curves (see also Grabov, 1990) shows that the voltage-gated K^+ channels were the major pathway for the rectifying currents across the plasma membrane of *L. stoloniferum* root hairs.

The following results indicate that the K^+ channels dominate in the mediation of the inward rectifying currents (which may represent an influx of positive charges or efflux of negative charges): (a) lack of the inward current in K^+-free medium even in the presence of 10 mM $CaCl_2$ and 10 mM NaCl (Fig. 7; note that concentration of Cl^- in this medium was 30 mM, as in control); (b) complete inhibition of inward current by CsCl (Fig. 9); (c) stimulation of the current after addition of KCl; (d) lack of an effect on inward current after addition of LiCl or NaCl (Fig. 9); (e) shift of the reversal potential of tail currents to the range of E_K after a hyperpolarizing pre-pulse (Grabov, 1990); (f) partial (52%) block of the inward rectifier by TEA (Fig. 4).

The arguments for domination of K^+ efflux in overall outward rectifying current (which may represent an efflux of positive charges or influx of negative charges) are: (a) the reversal potential of tail currents after a depolarizing pre-pulse was close to E_K at 2.5 and 0.25 mM K_2SO_4 in the medium (Grabov, 1990); (b) after a depolarizing pre-pulse the reversal potential of the tail currents became more negative by 65 mV upon replacement of K^+ with Na^+ in the medium (Grabov, 1990); (c) TEA blocked outward currents by 65% and inhibited the conductivity at −20 mV by 83%; (d) outward current was suppressed by addition of KCl, NaCl, LiCl, or CsCl (Fig. 9).

Neither inward nor outward current was changed after replacement of SO^-_4 with Cl^- or NO^-_3 in the medium (Grabov, 1990).

A similar system of inwardly and outwardly rectifying potassium channels and corresponding *I/V* profiles was described earlier by Schroeder (1988) for *Vicia faba* guard cell protoplasts.

The selectivity of the outward rectifier in the plasmalemma of *L. stoloniferum* root hairs was 13:1 for K^+ over Na^+ as calculated from reversal potentials in instantaneous *I/V* curves (Grabov, 1990). However, the external binding site of this channel is probably of low specificity, since the effect of K^+ on the outward current was only a little stronger than those of other alkali cations (Fig. 9A). The outward rectifier was practically insensitive to external Cs^+, resembling in this aspect the BK channels of animal cells (Ishikawa and Cook, 1993), which are voltage- and Ca-activated K channels of secretory cells (with a conductivity of 250 pS).

The selectivity for the inward rectifier was: $K^+(1) > NH_4^+(0.25) > Rb^+(0.19) > Na^+(0.15) > Li^+(0.04) > Cs^+(0.01)$

as demonstrated earlier (Grabov, 1990). The correlation between the shift of the trans-plasmalemma potential (Fig. 8) and the corresponding change in the inward currents after addition of an alkali cation (Fig. 9A) suggests that the inward rectifier is involved in setting the membrane voltage (Blatt, 1991). However, the high resting potential across the plasma membrane of *L. stoloniferum* root hairs (Fig. 2A) is evidence for a domination of an active pump at the voltages where K^+ channels were presumably silent (approximately −160 to −60 mV).

At hyperpolarization and depolarization, the K^+ channels become a dominant pathway due to the voltage-dependent activation. This property enabled us to diminish the interference from other transport systems in the study of K^+ channels, because we analyzed the trans-membrane currents at extreme voltages up to −200 mV of hyperpolarization and −20 mv of depolarization. The physiological relevance of the activation of potassium channels by hyperpolarization and depolarization has been discussed by Schroeder (1988), Blatt (1991), and Grabov (1990).

Because TEA is a highly selective blocker, we attributed the TEA-sensitive currents (Fig. 6A) to the K^+ channels. However, the K_D of TEA for K^+ channels in the plant plasma membrane depends on external K^+ (Tester, 1988) and may be rather large at high potassium content in the medium (for instance, 15 mM at external 5 mM K^+ for the outward rectifier; Sokolik and Yurin, 1986). The only partial (65%) blockade of outward rectifier by 10 mM TEA (Fig. 3) may be a consequence of high external K^+ concentration. The K_D of TEA for the inward rectifier was usually larger (Sokolik and Yurin, 1986), and the smaller blockade of inward rectifier (50%, Fig. 3) is in agreement with this fact. Thus, the TEA-sensitive currents represent a portion of the overall K^+ currents mediated by K^+ channels.

The lack of blockade of conductivity near E_K (zero K^+ current) is additional evidence that K^+ channels were the major targets for TEA. Nevertheless, TEA may evoke weak side effects. It was shown earlier that TEA may introduce a small linear leak in addition to its effect on K^+ channels (Blatt and Clint, 1989). Smith and Kerr (1987) also observed an increase in I after addition of TEA under some experimental conditions. They ascribed this effect to TEA^+ travel across the plasma membrane. We did not observe an increase in conductance after addition of TEA at any voltage (Fig. 5). However, the small depolarization after the addition of TEA (Fig. 4A) and consequent disagreement between E_K and reversal potential of TEA-sensitive currents (Fig. 6A) may indicate TEA^+ uptake. Thus, the blockade of conductivity by TEA may be undervalued at least at the voltage where K^+ channels demonstrated low activity (−160 to −60 mV).

Modulations of K^+ Currents by HCF III

The fact that HCF III affected both outwardly and inwardly rectifying currents (Figs. 2 and 3) suggests that potassium channels are modulated by the electron acceptor. Indeed, the K^+ channel blocker TEA efficiently inhibited HCF III-dependent current (Fig. 6B). From another point of view, the nonlinear TEA-sensitive current that was attributed to the potassium channel was strongly modulated by HCF III (Fig. 6A); outward current was inhibited but the inward current was activated.

Because HCF III failed to stimulate inward current in the absence of K^+ (Fig. 7) or after addition of Cs^+ (Fig. 9A), we assume that K^+ channels were also implicated in TEA-insensitive activation of inward current by the electron acceptor (Fig. 6). This effect accounts for the incomplete block of the inward rectifier by TEA.

The origin of the TEA-insensitive portion of HCF III-dependent outward current (30% at −20 mV, Fig. 6A) is not yet clear. Since the blockade by TEA may be incomplete, it is likely that this current was also mediated by the K^+ channels.

Since HCF III did not affect the channel selectivity (Figs. 7 and 8) and the effect of HCF III on K^+ current was reversible (data not shown), we suggest that (a) application of this electron acceptor did not result in any structural disturbance of the plasma membrane and (b) it modified the channel activity within a physiological range.

Recently, stimulation of the inward TEA-independent potassium current by HCF III and HCF II was observed by Thiel and Tester (1990) in *Chara corallina*. Some discrepancy between our data and theirs may result from differences between the transport systems of algae and higher plants. However, *Chara* cells were pretreated by electrical pulses, transferring the cells into the so-called K state, whereas high electrogenic activity was typical for root hairs (−145 mV at 10 mM external K^+) in our experiments. Hence, the pattern of response may also be determined by the state of the cell.

HCF III-Evoked Depolarization Does Not Originate from Stimulation of the Inward Rectifier

The increase in inward K^+ current may allow the dissipation of the electrical component of the proton motive force created by the H^+ pump across the plasma membrane, giving rise to a membrane depolarization. This is clearly demonstrated by the correlation between the inward currents and the E_m at different ionic compositions of the medium in the absence of HCF III (Figs. 7, 8, and 9A). Surprisingly, however, the change in free-running E_m did not correlate with the change in inward current after addition of HCF III: (a) Depolarization of the plasma membrane occurred practically immediately after application of HCF III, whereas the inward rectifier was completely activated only after approximately 90 min (Fig. 7). (b) The membrane was largely depolarized even in K^+-free medium, whereas no activation of inward rectifier occurred at these conditions. (Fig. 7).

We also did not observe any signs of activation of potassium channels at free-running E_m after addition of HCF III. Membrane G was practically not changed (Fig. 3). The plasma membrane K^+/Na^+ selectivity after addition of HCF III was even decreased from 3.4 to 1.6, as can be calculated from the data in Figure 7A with the use of Goldmann equation (Hille, 1972). Therefore, we suggest that membrane depolarization was evoked by other processes distinct from activation of the K^+ channel. This depolarization should inactivate the voltage-gated inward rectifier compensating HCF III-induced activation.

Since the effect of HCF III on the membrane potential was

instantaneous and repolarization occurred immediately after HCF III removal (Figs. 2 and 4) and because of the high capacity of *L. stoloniferum* root cells for HCF III reduction (Grabov, 1993), we assumed that depolarization resulted from trans-plasma membrane electron transfer (Ivankina and Novak, 1988; Döring et al., 1990; Ullrich and Guern, 1990). The small change in membrane current within the range of the free-running potential may reflect the modification of electron flux evoked by HCF III (Figs. 2 and 4B). However, this change was close to the limits of detection in the present experiments (see also Thiel and Tester, 1990). Theoretically, upon the addition of HCF III the subsequent depolarization may also be due to an activation of Ca^{2+} uptake or Cl^- efflux; however, we do not have any experimental evidence for this.

Putative Mechanisms of K^+ Channel Regulation by Plasmalemma Redox Reactions

The time course of inward rectifier activation after addition of HCF III (Fig. 7) suggested that it was a secondary effect probably resulting from a cascade of regulatory events. Apparently, the ion channels should have a central role in signaling due to the large amounts of ionic charge that they may transport across the plasma membrane within a short time (Brown, 1993). However, it is not clear what the signal for channel modulation is in our case.

H_2O_2, a potential product of the plasmalemma redox reactions (Penel and Castillo, 1991), was shown to serve as second messenger in the transduction of the defense signal in plants (Apostol et al., 1989). Active oxygen species were also demonstrated to be involved in regulation of potassium channels during stress response of human cells (Kuo et al., 1993). Thus, administration of HCF III may influence the production of active oxygen species and affect potassium channels by means of these messengers.

Several sulfhydryl-group reactants have been shown to modify the properties of potassium channels: DTT and 2-mercaptoethanol in cation channels of yeast vacuole (Bertl and Slayman, 1990) and of *Arabidopsis* plasma membrane (Spalding et al., 1992) and *p*-chloromercuribenzenesulfonic acid in voltage-dependent K^+ channels of *Chara* plasma membrane (Thiel, 1991). Because one of the functions proposed for the "standard" system is to keep sulfhydryl groups on the outside of the plasma membrane in the reduced state (Bienfait and Lüttge, 1988), the modulation of plasmalemma redox reaction by external electron acceptors may also exert an influence on potassium channels through a modification of sulfhydryl groups.

Taking into account that cytoplasmic pH may also serve as second messenger in plants (Felle, 1989), it is not excluded that the change of cytoplasmic pH after application of HCF III (Ullrich and Guern, 1990; Grabov et al., 1993) may be a step in a signal-transduction cascade leading to modification of potassium channels.

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