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Energy-linked Potassium Influx as Related to Cell Potential in Corn Roots[1]

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ABSTRACT

Cell potentials and K^+ (^{86}Rb) influx were determined for corn roots over a wide range of external K^+ activity (K°) under control, anoxic, and uncoupled conditions. The data were analyzed using Goldman theory for the contribution of passive influx to total influx. For anoxic and uncoupled roots the K^+ influx shows the functional relationship with K° predicted with constant passive permeability, although K^+ permeability in uncoupled roots is about twice that of anoxic roots. In control roots the equation fails to describe K^+ influx at low K°, but does so at high K°, with a gradual transition over the region where the electrical potential becomes equal to the equilibrium potential for K^+ ($\psi = E_K$). In the low K° range, where net K^+ influx is energetically uphill, participation of an energy-linked K^+ carrier is indicated. In the high K° range, K^+ influx becomes passive down the electrical gradient established by the cell potential. Since the cell potential includes a substantial electrogenic component, anoxia or uncoupling reduces passive influx.

In a previous paper (3) we described a mathematical analysis for the electrogenic "pump" potential of corn root cells as a function of external K^+ activity. Experimentally, ψ_p[2] was analyzed as the difference in electrical potential between coupled and uncoupled cells; that is:

$$\psi_p = \psi - \psi_D$$

where ψ_D was the diffusion potential found after uncoupling. The assumption was made that uncoupling did not affect ionic permeabilities.

The analysis showed that ψ_p rapidly declined with increasing K° up to 0.7 mM, the mechanism I range of high affinity carrier transport (6). Beyond this point, in the low affinity mechanism II range (1–50 mM), ψ_p increased, extrapolating to a constant value at very high K°. For the low K° range we suggested that progressive saturation of a K^+ carrier linked to an electrogenic system, possibly an H^+/K^+-ATPase (9, 14), would increase the inward current of K^+, decrease the net electrogenic efflux, and thus produce the observed depolarization. In the upper K° range the increase in ψ_p may reflect K^+ stimulation of ATPase activity (10, 12) and/or allosteric regulation of the K^+ carrier (7).

Except for a partial study by Mertz and Higinbotham (18) with barley roots there are no data analyzing K^+ influx over a wide range of K^+ from the electrophysiological viewpoint. There is a lack of information on conditions under which K^+ influx primarily represents passive ion movement down an electrical gradient, controlled by those factors commonly pooled under "permeability" (P_K). This passive influx corresponds to electrophoretic entry of K^+, or in chemiosmotic terminology, entry via a uniport (20).

To the extent that such K^+ influx exists the passive flux equations developed following Goldman should hold (2):

$$\overrightarrow{\phi_K} = -\frac{P_K F}{RT} \cdot \frac{\psi K^\circ}{1-\xi} \qquad (1)$$

where $\phi_k = K^+$ influx and $\xi = \exp(F\psi/RT)$. With P_K a constant the following transformation can be made:

$$\frac{\overrightarrow{\phi_K}(1-\xi)}{\psi} = -\frac{P_K F}{RT} K^\circ = -\eta_K K^\circ \qquad (2)$$

where in an isothermal system, η_K = constant. Assuming no changes in surface area, η_K can be calculated using influx/g fresh weight data with the understanding that it incorporates an unknown weight to surface area ratio. If η_K calculated from influx and potential data over a range of K° proves not to be constant, there either must be changes in P_K or the influx is not driven only by the electrical gradient.

We have made this analysis for low salt, 4-h washed corn root tissue under normal (aerated), anaerobic, and uncoupled conditions. In the lower K° range, where $\psi < E_K$, the data indicate that K^+ influx requires the participation of an energy-linked carrier system. In the upper K° range, where $\psi > E_K$, K^+ influx becomes passive down ψ, which, however, includes a significant electrogenic component. By using anoxia to eliminate the energy-linked electrogenic mechanism without resorting to chemicals which may interact with the membrane, it is indicated that uncoupling with FCCP does in fact alter P_K.

MATERIALS AND METHODS

Methods for raising corn seedlings (*Zea mays* L., hybrid [A 619 × Oh 43] × A632 Crows Hybrid Corn Co., Milford, Ill.) and cutting and washing root segments were those previously described (8). The basic medium for washing and all experimental determinations was 0.2 mM $CaCl_2$ + 0.2 mM Mes buffer adjusted to pH 6.0 with Tris. K^+ to the desired level was added as K_2SO_4. K^+ influx solutions were labeled with ^{86}Rb to a minimum of 20,000 cpm/ml.

Electrical recordings were made as described (3) but at 28 to 30 C; this produced no change in control potentials from those previously reported. For anoxia recordings, potentials were first

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[2] Abbreviations: ψ, ψ_D and ψ_p: total, diffusion and electrogenic cell potentials; K° and K^i: potassium activity outside and inside the cell; P_K: permeability coefficient for K^+; E_K: Nernst equilibrium potential for K^+; FCCP: *p*-fluoromethyoxy-carbonyl cyanide-phenylhydrazone.

determined in aerated medium, then N_2 bubbling was begun in the solution reservoir. For our tissue holder (0.5-ml volume), a flow rate of about 5 ml/min with a solution depth above the tissue of 2 to 3 mm was critical to producing stable, maximally depolarized potentials.

K^+ (^{86}Rb) influx was measured in roots prepared and washed as for electrical recordings except that the 2-cm root segments were cut in half. For treatments in which $K^\circ < 0.2$ mM, the concentration during washing, the roots were rinsed for 10 min in K^+-free medium. Forty 1-cm segments weighing about 0.4 g were placed in 100 ml of aerated uptake solution for control and FCCP treatments. After 10 min of uptake the solution was poured through cheesecloth, which was then tied into a bag around the segments, followed by a brief rinse in ice-cold deionized H_2O and removal of nonaccumulated ions by 15 min exchange in ice-cold 5 mM $CaCl_2$ + 1 mM KCl. The high Ca^{2+} concentration facilitates complete desorption (5). FCCP treatments (10 μM, 0.5% ethanol) were started in the washing solution during the last 30 min, and the same concentration was maintained in the K^+-free rinse and during uptake.

In order to facilitate the attainment of anoxia the segments were placed in 20 ml of medium in 25-ml Erlenmeyer flasks. N_2 bubbling was begun, and after 5 min the label was added. An O_2 electrode showed 4 min of N_2 bubbling adequate to reduce O_2 to <1% of aerated solution.

For determinations of η_K by linear regression, the slope of the line forced through the origin was determined statistically as $\eta_K = \Sigma xy/\Sigma x^2$ (24). The intercept with both anoxia and FCCP was not statistically different from the origin.

RESULTS

Potentials *versus* K°. The K° dependence of ψ under anoxia is given in Figure 1, along with previously reported data for control and uncoupled roots (3). ξ-analyses of the data for anoxia and uncoupling give linear plots characteristic of Goldman diffusion potentials (Fig. 2), with intercepts at $\xi = 0$ which are similar (ρ_{Cl} Cl^i, Table I). The difference in slope, which indicates apparent K^i (Table I), is quite large and in terms of the analysis accounts for the difference of approximately 35 mv, K°-independent, in the two values for ψ_D (Fig. 1). Apparently, ψ_D can be a function of the means used to reveal it. This is also indicated by the data of Anderson *et al.* (1).

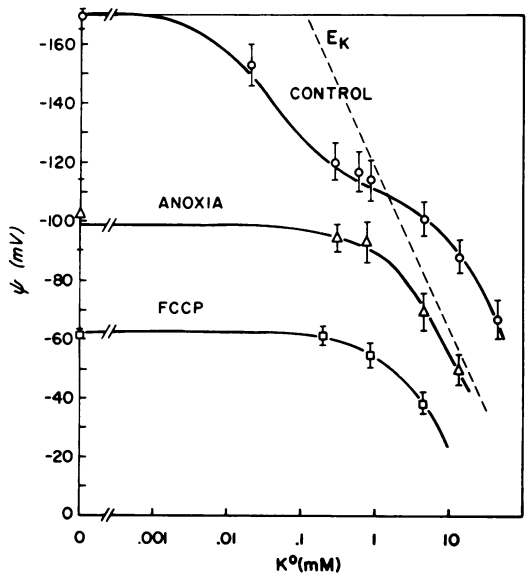

FIG. 1. Potential *versus* K° for control, anoxia, and FCCP uncoupled root cells. Solid lines are drawn according to the equation and parameters in Table I. Error bars are SD of experimental values. Dashed line is E_K based upon K^i = 110 mM.

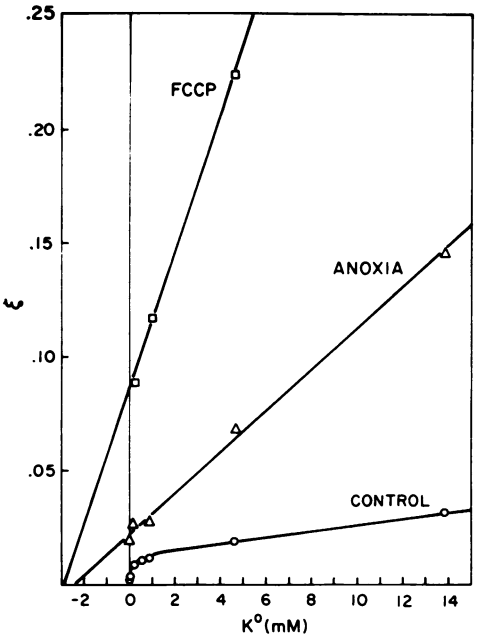

FIG. 2. ξ *versus* K° corresponding to data in Figure 1. Parameters describing the curves are given in Table I. FCCP and anoxia lines show linear relationships predicted by the Goldman equation: 1/slope = K^i and $K^\circ = -\rho_{Cl} \cdot Cl^i$ at $\xi = 0$ (see ref. 3).

Table I. *ξ-Parameters for Control, Anoxic, and Uncoupled roots*

$$\Psi = \frac{RT}{F} \ln \xi = \frac{RT}{F} \ln \left[\frac{K^0 + A}{K^0 + B} \right] \cdot (\alpha K^0 + \beta) \quad \text{(from ref. 3)}$$

	A	B	α	β	K^{ia}	$\rho_{Cl}Cl^{ib}$
Control	0.0108	0.1090	0.00132	0.0135		
Anoxia			0.0091	0.0222	110	2.4
10 μM FCCP			0.0303	0.0874	33	2.9

[a] Calculated as the inverse of the slope of ξ *versus* K^0.
[b] Calculated as the *x* intercept of ξ *versus* K^0.

Analysis gave a mean concentration of 36 mM K^+ for tissue water (3), which largely reflects vacuolar content. The vacuole has been suggested to have a positive potential of 10 to 20 mv with respect to the cytoplasm (15, 19, 22, 25), which if true would be consistent with the high K^+ content in the cytoplasm indicated by anoxia (Table I). Perhaps FCCP has eliminated K^+ compartmentation, giving estimated K^i values more typical of the average concentration. There is a factor of unknown importance here, in that uncoupling appears to be more effective than anoxia in reducing ATP levels (13). However, in our experiments anoxia was very effective in reducing active K^+ influx (Table II and following discussion).

Flux Analysis. Table II gives K^+ influx for control, anoxic, and uncoupled roots, and the values of η_K determined from equation 2 by linear regression. Figure 3 plots the calculated K^+ influx along with the experimentally determined values. Both anoxia and uncoupling, which eliminate the energy-linked component of K^+ influx, produce regressions of the form expected for passive influx. The greater influx under uncoupled conditions than under anoxia, in spite of the lower ψ, is due to η_K (and thus P_K) being about twice the anoxic value (Table II). On the other hand, there must be proportionate changes in ρ_{Cl} (or Cl^i) since the $\rho_{Cl}Cl^i$ values are about equal (Table I).

Figure 4 illustrates graphically the effects of anoxia on K^+ influx compared to control tissue, and plots η_K calculated at each value of K°. At high K° the values of η for control and anoxic

Table II. *K^+ (^{86}Rb) Influx into 4-h Washed, Low Salt Corn Root Segments As a Function of K^0 for Control, Anoxic and Uncoupled Conditions, and Respective η_K Values Determined by Linear Regression*

K^0	K^+ (^{86}Rb) Influx		
	Control	Anoxia	FCCP
mM	μmol/g fresh wt·h ± SD		
0.002	0.42 ± 0.12 (7)[a]	0.0033 ± 0.0005 (4)	0.0026 ± 0.0003 (4)
0.02	2.50 ± 0.78 (2)	0.024 ± 0.003 (4)	0.027 ± 0.0015 (4)
0.2	4.56 ± 0.40 (4)	0.128 ± 0.004 (3)	0.227 ± 0.016 (4)
0.86	5.00 ± 0.52 (4)		
4.59	6.77 ± 0.55 (8)	2.23 ± 0.25 (3)	3.62 ± 0.20 (4)
13.8	10.7 ± 1.3 (4)	4.74 ± 0.08 (3)	6.32 ± 0.41 (4)
29.0	13.0 ± 1.4 (5)		
η_K		0.0059	0.0130

[a] ( ): No. of determinations.

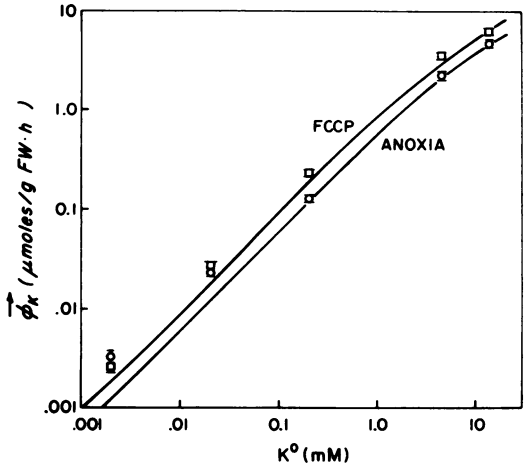

FIG. 3. K^+ influx *versus* K° for FCCP uncoupled and anoxic roots. Solid lines were drawn using equation 2 with η_K determined by linear regression and ψ calculated from Table I.

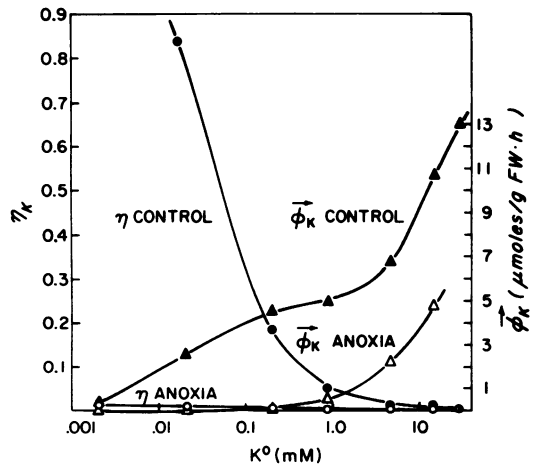

FIG. 4. K^+ influx *versus* K° for control and anoxic roots and values of η_K calculated for each separate value of K°.

roots are similar, but there is a very large escalation of control η_K in the low K° range. There is no basis for attributing this rise in η_K to increased passive permeability as K° falls. It is a reflection of the energy-linked net influx of K^+ which occurs at low K° in low salt roots, and which is illustrated by the difference in control and anoxic K^+ influx at low K°. In this range K^+ influx is primarily active; that is, $\psi < E_K$ (Fig. 1) and influx is against the thermodynamic gradient. In this circumstance the passive flux equation would not be expected to hold.

Figure 5 shows the active and passive components of the total K^+ influx. Passive influx was calculated using $\eta_K = 0.0059$ determined for anoxic roots; this is similar to the value of 0.0055 for control roots at $K^\circ = 29$ mM. Active influx is that portion of the total which cannot be accounted for as passive. It should be noted that "passive" influx does not mean there is no energy-linked component to the influx (compare influx under anoxia, Fig. 4, with passive influx, Fig. 5); any energy-linked mechanism which increases ψ will increase passive influx (equation 1).

The curve for total flux in Figure 5 illustrates the transition which is characteristic of the dual isotherm phenomenon of kinetic analysis (6). It is probably relevant that this transition occurs in the range where ψ becomes more negative than E_K, where net influx becomes energetically downhill, and where passive influx begins to make a significant contribution. The indicated inactivation of carrier-mediated, active influx as K° increases is reasonable in view of the magnitude of the passive influx. At the unphysiological concentration of 50 mM ($K^\circ = 29$ mM) all of the K^+ influx is accounted for as passive influx down the electrical gradient.

DISCUSSION

Our intent in this study was to explore the possible relationship between the systems responsible for the postulated dual transport mechanisms of ion absorption (6) and the correlated changes in ψ_p. For this purpose we have calculated for a wide range of K° that fraction of the K^+ influx which is attributable to passive movement in response to the electrical gradient, using data on ψ and K^+ influx *versus* K° under control, anoxic, and uncoupled conditions. Contrary to our previous assumption (3) the strong uncoupling action of 10 μM FCCP does appear to increase P_K somewhat, and may also break down K^+ compartmentation. Although there is no standard by which to rule out some effect of anoxia on P_K, the essential identity of η_K values of control and anoxic tissue at high K° suggests that the anoxic value of η_K is a good estimate of normal permeability.

The analysis indicates that in the range of K° where $\psi \simeq E_K$ (0.5–2 mM, depending on estimates of K^i at the plasmalemma), and where ψ_p is minimal (3), there is a transition from active to passive K^+ influx which correlates with the change from mechanism I to mechanism II. Although our study is not applicable to much of the debate which has surrounded the kinetic studies of

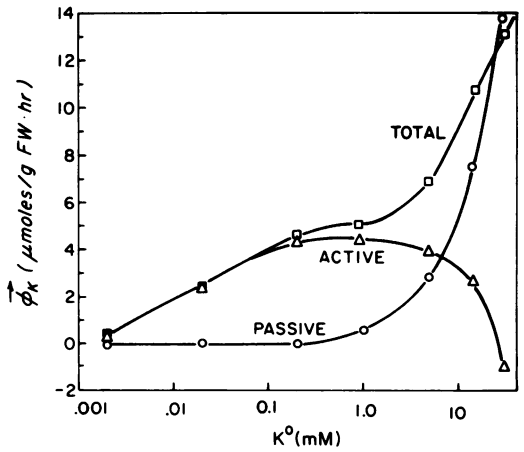

FIG. 5. K^+ influx *versus* K° in control roots resolved into passive and active components. Total influx is replotted from Figure 4. Passive is calculated from equation 2 with $\eta_K = 0.0059$. Active is the algebraic difference.

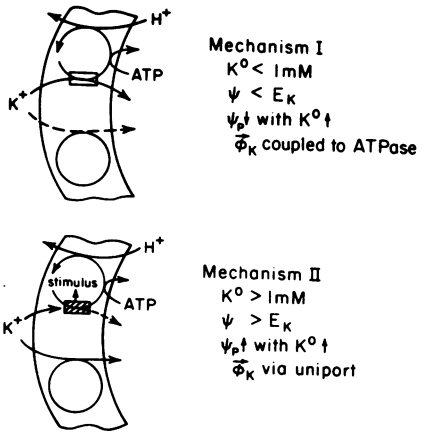

FIG. 6. Schematic model of electrogenic ATPase function and K^+ influx at the plasmalemma of corn root cells under low and high K° (mechanisms I and II of kinetic analysis). See text for description.

dual or multiple isotherms (see refs. 6 and 21 for recent reviews), it does indicate that electrical parameters are important in the kinetics of K^+ influx, and that there is some change in the electrogenic system between mechanisms I and II.

Figure 6 schematically models these mechanisms as we presently envision them. At low K°, we propose that K^+ influx is linked to an H^+-extruding ATPase of the plasmalemma, which in the recovery phase carries bound K^+ inward, much as previously suggested by this laboratory (9, 14) and others (4, 10, 16, 17, 23). The system is electrogenic to a degree inversely proportional to the availability of K^+ to the carrier enzyme, lowering ψ_p to a minimum at about the electrical crossover point where $\psi = E_K$ (3). This carrier system produces active K^+ transport; that is, net K^+ influx against the thermodynamic gradient. In chemiosmotic terms, it might be considered an energy-linked H^+/K^+ antiport of variable stoichiometry, somewhat as suggested by Poole (17, 23).

As K° increases into the mechanism II range, we propose a gradual substrate inhibition of carrier function, but with a concomitant stimulation of the ATPase activity by K° as reported by Hodges and colleagues (10), and demonstrated for corn plasmalemma preparations (11, 12). Thus, the K^+-carrier of the ATPase is suppressed while the H^+-extruding function is increased, giving the noted hyperpolarization of ψ_p (3). K^+ influx in the mechanism II range becomes primarily a passive electrophoretic penetration through a uniport. Obviously, the kinetic constants would be changed, and might even show a series of discontinuities as active influx declines and passive influx increases.

Some qualification of the model may be needed; there may, for example, be separate H^+-extruding ATPases (or electron transport chains) for the two K° ranges. Such changes, however, would not alter the principles disclosed by the analysis—at low K°, K^+ is "carried" in by a respiration-linked transport mechanism, and at high K° it enters passively down an energy-linked electrical gradient.

In general, our conclusions are close to those reached by Glass and Dunlop (7) from studies of K^+ influx into low and high salt roots. They found K^+ influx in the mechanism I range to be suppressed by high K°, but progressively less so as K° increased into the mechanism II range, where K^+ influx was considered to be passive. They suggest that passive K^+ influx might not be completely independent of a metabolic input, such as that contributing to the electrical potential difference (see discussion and citations, ref. 7). The analysis we have made here on passive K^+ influx as a function of cell potential gives experimental support to this suggestion. Our results, however, also suggest that high external K° can also serve to repress mechanism I.

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