Mathematical Analysis of the Dependence of Cell Potential on External Potassium in Corn Roots[1]

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ABSTRACT

The K^+ dependence of normal (ψ) and diffusion (ψ_D) potentials in corn roots [*Zea mays* L., hybrid (A619 × Oh43) × A632] was determined experimentally and analyzed with respect to the parameter ξ [defined as exp (F ψ/RT)]. In the presence of 10 micromolar carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP), ψ behaved as expected of a diffusion potential. Based upon the assumptions (a) that FCCP did not change any term of the Goldman-Hodgkin-Katz equation, and (b) that total potential was functionally the algebraic sum of ψ_D and ψ_P (the deviation from ψ_D due to an electrogenic system), ψ_P was found to be a complex function of external potassium and to have a minimum value of 0.69 millimolar K ion activity outside the cell. Analysis of ψ allowed us to develop an equation which predicts a complicated K^+ dependence of ψ such as that found by Mertz and Higinbotham (Membrane Transport in Plants and Plant Organelles. Springer-Verlag 1974).

Following the great rise in popularity of the chemiosmotic hypothesis and noting the great complexity of the transport data across higher plant cell membrane systems, Mitchellian transport systems have been suggested by several investigators (*e.g.* 3, 10, 11, 16). A general model suggested by Hanson (6) includes three types of transport: (*a*) active proton efflux which is electrogenic, probably associated directly with activity of an ATPase and possibly coupled closely to active K uptake (see also refs. 1 and 11); (*b*) electrophoretic movement (uniport); and (*c*) neutral exchange systems making use of the chemical gradient for protons. To date, however, no single element of the system has been characterized in such a way as to be useful to the model. The object of the present study was to characterize changes in ψ which occur over a range of external K activities. This is a first step in understanding and separating the electrically non-neutral elements of the models.

We have found it useful to analyze $\xi \equiv \exp(F\psi/RT)$. As a result we have been able to describe mathematically the behavior of ψ as a function of K°; to show that in the presence of 10 μM FCCP,[2] ψ behaves as expected of a diffusion potential; and to characterize ψ_P, the deviation of ψ from ψ_D due to an electrogenic system.

MATERIALS AND METHODS

TISSUE PREPARATION

Methods for raising corn seedling [*Zea mays* L., hybrid (A619 × Oh43) × A632] were previously described (8). All recordings

were done on cells in the first cortical layer in the 0.5- to 2.5-cm segment of the primary root of 3-day-old-plants. Only segments lacking root hairs were used. Segments were washed for 4 hr prior to recording, 10 segments to 50 ml solution at 30 C. The washing medium contained (concentrations in mM): $CaCl_2$, 0.2; MES, 0.2; K_2SO_4, 0.1; with pH adjusted to 6.0 with Tris. During recordings of cell potentials the same solution was used with K^+ content adjusted to the desired level by alteration of K_2SO_4 concentration. Temperature during recordings was approximately 23 to 25 C. Recording circuitry was that described by Mertz and Arntzen (12). Measured potentials were considered to exist entirely across the plasmalemma, *i.e.*, with no potential difference across the tonoplast (14).

MINERAL ANALYSIS

Total K^+, Na^+, and Cl^- were determined by hot water extraction of the tissue. K^+ and Na^+ were assayed using a flame photometer (model 143; Instrument Laboratories, Inc., Lexington, Mass.). Cl^- was assayed using either a Fiske Chlor-o-counter (Fiske Associates, Inc., Uxbridge, Mass.) or an Aminco-Cotlove chloride titrator (American Instrument Co., Silver Spring, Md.). Average tissue water content was found to be approximately 95%. Vacuolar ion concentrations were calculated by assuming that all of the extracted ions were from the vacuole and that the vacuole contained 80% of the total tissue water (14). Correcting to activity based upon KCl, average vacuolar contents were K^+, 35.6 mM, Na^+, <5 mM, and Cl^-, 14.1 mM. (Throughout this paper, conversion of concentration to activity is based upon Table 2.1 in ref. 15).

MATHEMATICAL ANALYSIS

The ξ-Function. Equation 1 is the Goldman-Hodgkin-Katz equation (7) defining the diffusion potential:

$$\psi_D = \frac{RT}{F} \, \ell n \, \frac{K^\circ + \rho_{Na} \, Na^\circ + \rho_{Cl} \, Cl^i}{K^i + \rho_{Na} \, Na^i + \rho_{Cl} \, Cl^\circ} \tag{1}$$

where K°, etc. are the ionic activities, ($^\circ$, or inside, ^{i}); ρ_j is the permeability ratio of species j to potassium; and R, T, and F have their usual meanings. From this equation it is obvious that ψ_D is not a linear function of K° nor of the logarithm of K°. Rather, if K° is varied with a relatively impermeant ion such as SO_4^{2-}, the slope of ψ_D *versus* log K° approaches zero when $\rho_{Na} \, Na^\circ + \rho_{Cl} \, Cl^i \gg K^\circ$, and approaches 2.303 RT/F when the inequality is reversed (Fig. 1a). This characteristic is often overlooked in the plant literature. The observation by Mertz and Higinbotham (13) of a slope of 58 mv in the 10 μM K° region is a much more significant indication of non-Goldman behavior than are slopes of less than 58 mv at much higher external K^+ levels.

When considering a single ionic species such as K, the visual presentation can be linearized and the search for deviations facilitated by plotting instead the function described by equation 2:

$$\xi_D = \exp (F\psi_D/RT) = \frac{K^\circ + \rho_{Na} \, Na^\circ + \rho_{Cl} \, Cl^i}{K^i + \rho_{Na} \, Na^i + \rho_{Cl} \, Cl^\circ} \tag{2}$$

[2] Abbreviations: FCCP: carbonyl cyanide p-trifluoromethoxyphenylhydrozone; K°, K^i, Cl^i: ion activities outside ($^\circ$) and inside (^{i}) the cell.

In the case of corn, a sodium excluder, and in these experiments absent from the growth and recording media, Na terms can be omitted. Further, since $Cl^o \ll K^i$ (about 1% in these experiments), it is also reasonable to omit the term $\rho_{Cl} Cl^o$.

Equation 2 can therefore be simplified:

$$\xi_D = \frac{1}{K^i} \cdot K^o + \frac{\rho_{Cl} Cl^i}{K^i} \tag{3}$$

In addition to its linearity with respect to K^o this form is useful in that it allows one to estimate K^i as the reciprocal of the slope and $-\rho_{Cl} Cl^i$ as the x intercept.

For consideration of the electrogenic component, ψ_p, and the total potential, ψ, two further assumptions are required: (a) that the methods for revealing ψ_D, e.g. application of FCCP, do not change K^i, Cl^i or ρ_{Cl}; (b) that the total potential is functionally the algebraic sum of the diffusion potential and an electrogenic component, i.e. that:

$$\psi = \psi_D + \psi_p \cdot \tag{4}$$

Then:

$$\exp (F\psi/RT) = \exp (F(\psi_D + \psi_p)/RT) \tag{5}$$

$$= \exp (F\psi_D/RT) \cdot \exp (F\psi_p/RT)$$

or

$$\xi = \xi_D \cdot \xi_p = \frac{K^o + \rho_{Cl} Cl^i}{K^i} \cdot \xi_p = \frac{\xi_p}{K^i}(K^o + \rho_{Cl} Cl^i) \tag{6}$$

Note that if ψ_p is constant, the slopes of ξ_D and ξ as a function of K^o will differ by a constant multiplier (ξ_p), but will extrapolate to the same x intercept, $-\rho_{Cl} Cl^i$. If, as is the case in this study, the x intercepts are different, or if the slope is not constant, ξ_p (and thus ψ_p) is not a constant. Based upon our first assumption above, i.e. that $\rho_{Cl} Cl^i$ is constant, analysis then requires a technique for determining ξ_p.

Empirical Techniques. Figure 2 shows that values of ψ gave a plot of ξ with these characteristics: (a) apparently linear over K^+ activities of 4.5 to 45 mM, but not extrapolating to the same x intercept as ξ_D; (b) nonlinear below about 4.5 mM, and with a positive value at $K^o = 0$; that is, $\psi \neq -\infty$.

We found one way to describe closely the deviation from nonlinearity at low K^o was to assume that the curve approached linearity in a fashion similar to that of first order (Michaelis-Menten) enzyme kinetics.

Since the curve (Fig. 2) showed a finite value for ξ at $K^o = 0$, it was necessary for mathematical analysis to adjust the curve by a constant A (in effect, a y axis shift). This produces the following equation for the "proportional saturation" of the system with K^+:

$$\chi = \frac{K^o + A}{(K^o + A) + b} \tag{7}$$

where A is the deviation of the experimental curve from $K^o = 0$ at the x intercept; and b is the affinity constant of the system for K^+ (i.e. the equivalent of K_m). As an empricial tool:

$$\chi = \frac{\xi_{observed}}{\xi_{predicted}} = \frac{K^o + A}{K^o + B} \tag{8}$$

where ξ predicted = value predicted for ξ by extrapolation of the linear portion of the curve ($K^o > 4.5$ mM) and $B = A + b$. A and B become phenomenological constants, and were calculated by solving simultaneous equations using values of χ at $K^o = 0$ and $K^o = 0.19$ mM (where χ was still well below 1, and a large number of determinations of ψ had been made).

The experimentally determined values of ψ gave a linear regression for the higher values of K^o (Fig. 2) which did not extrapolate to the same x intercept as that for ψ_D (Fig. 1). Based on the first of our assumptions (above), $\rho_{Cl} Cl^i$ is to be considered constant

under all experimental conditions, and thus despite the apparent linearity, ξ_p is also not constant in the range $K^o = 4.5$ to 45 mM. This necessitated an empirical correction for the deviation in intercept.

From equations 3, 6, and 8 we can derive a formula describing the K^+ dependence of ξ. Together with the formula for ξ_D, this allows us to calculate ξ_p. The equations are rewritten in simple linear form:

$$\xi_D = \alpha_D (K^o + \beta_D) \tag{9}$$

$$\xi = \alpha (K^o + \beta) \tag{10}$$

where the α values are the slopes and $\alpha\beta$ values are the y intercepts. Thus in equation 9:

$$\alpha_D = \frac{1}{K^i}$$

and

$$\beta_D = \rho_{Cl} Cl^i$$

From this:

$$\xi_{observed} = \chi \cdot \xi_{predicted} = \chi \cdot \alpha (K^o + \beta) \tag{11}$$

and

$$\xi_p = \frac{\xi_{observed}}{\xi_D} = \chi \cdot \frac{\alpha(K^o + \beta)}{\alpha_D(K^o + \beta_D)} \tag{12}$$

$$= \frac{K^o + A}{K^o + B} \cdot \frac{\alpha(K^o + \beta)}{\alpha_D(K^o + \beta_D)}$$

Data Selection. Analyses for this paper were performed on data from six recordings of cell potential which were made over a period of 25 days (five recording sessions). All cells showed similar initial potentials in 0.2 mM K^+ medium ($K^o = 0.19$).

RESULTS

Diffusion Potential. Following introduction of 10 μM FCCP into the medium there was an immediate depolarization of cell potential which leveled out in about 15 min. To insure complete uncoupling we allowed another 15 min before beginning salt changes. Values of ψ_D were reproducible to within 2 mv at a given K^o in each experiment.

Figure 1a is a plot of ψ_D versus K^o. The corresponding plot of ψ_D is shown in Figure 1b. (Although these figures include no values of K^+-free solution, other experiments showed that changing from 0.2 mM to 0 mM K^+ resulted in a rapid hyperpolarization of a few mv. The new level was steady for at least 10 min, and return to 0.2 mM K^+ restored the initial level.) ξ-Analysis indicates by its linearity that the potential is behaving as would be expected
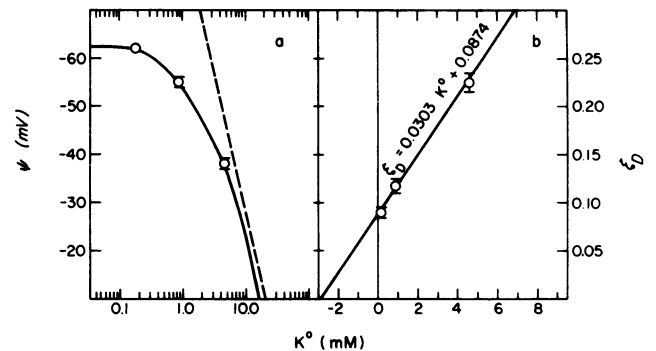

FIG. 1. a: K^+ dependence of diffusion potential as determined in the presence of 10 μM FCCP; dotted line has slope of 58 mv/log unit. b: Corresponding plot of ξ_D. Experimental points fall on the regression line $\xi_D = 0.0303 K^o + 0.0874$. Means and standard errors are plotted.

from a diffusion potential. From the slope K^i is indicated to be 33 mM, very close to the value of 36 mM determined from tissue extracts. Extrapolation of the line through $\xi_D = 0$ indicates that ρ_{Cl} Cl^i is 2.9 mM. If we accept a value of cytoplasmic Cl$^-$ approximately 20% higher than the measured internal Cl$^-$, i.e. 17 mM (14) this indicates that $\rho_{Cl} \doteq 0.17$. This is similar to the value of 0.19 calculated for flexor cells in *Phaseolus coccineus* by Gradmann and Mayer (5) though considerably higher than the value of 0.03 reported for *Avena* coleoptiles (14).

Total Potential. In normal media (no FCCP) changes in potential over the range $K^° = 0.19$ to 45 mM were completed in the time needed for a concentration change in the chamber, about 1.5 min. Some hysteresis was noted at low K^+ levels, and this was treated by determining ψ with increasing concentrations of $K^°$ over the 0.19 to 45 mM range (where there was little hysteresis), and subsequently in K^+-free medium. These data are plotted as ξ versus $K^°$ in Figure 2.

At high $K^°$, ξ versus $K^°$ is apparently linear. If ψ_p were constant in this range the line should extrapolate to -2.9 mM, as with ξ_D (Fig. 1b). That it extrapolates to -10.2 mM implies that either ρ_{Cl} is altered by FCCP, giving a false indication of the normal diffusion potential, or that ψ_p is in fact not constant over this range. We take the similarity of apparent diffusion potentials in the presence of various agents (10) to justify for now the assumption that ρ_{Cl} Cl^i is constant and that ψ_p is not.

Electrogenic Potential. The electrogenic component, ξ_p, was determined as the ratio ξ/ξ_D at each $K^°$ using equation 12. Using the linear equations in Figures 1 and 2, and the values of A and B determined at 0 and 0.19 mM $K^°$, equation 12 becomes

$$\xi_p = \frac{K^° + 0.0108}{K^° + 0.1090} \cdot \frac{0.00132 \ (K^° + 10.23)}{0.0303 \ (K^° + 2.88)}$$

Converting this back to potential $(\psi_p = \dfrac{RT}{F} \ln \xi_p)$ produces the curve for ψ_p versus $K^°$ (Fig. 3). At low K^+ the first term of equation 12 (χ) is dominant, producing a hyperbolic decline in ψ_p with increasing $K^°$. As $\chi \rightarrow 1$, the second term produces a slow rise in ψ_p. Only at very high K^+ activity will ξ_p (and ψ_p) become constant.

The applicability of this technique is illustrated in Figure 4. The circles represent our experimental values and deviate from the calculated curve by at most 4 mv. The curve is that described by equation 11 for ξ observed.

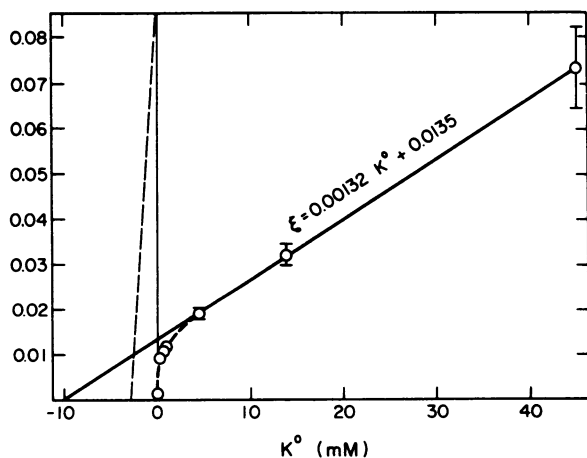$$\xi = \frac{K^° + 0.0108}{K^° + 0.1090} \cdot 0.00132 \ (K^° + 10.23)$$

FIG. 2. ξ-Plot of K^+ effects in normal cells. Regression line was determined on observations above 4.5 mM $K^°$. At lower values a hyperbolic curve was fitted to the approach of the dotted curve to the regression line. Dashed line describes ξ_D redrawn from Figure 1b.
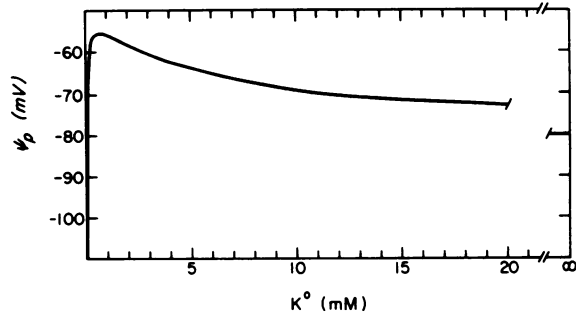

FIG. 3. K^+ dependence of electrogenic component of cell potential computed from equations for diffusion and total potentials.
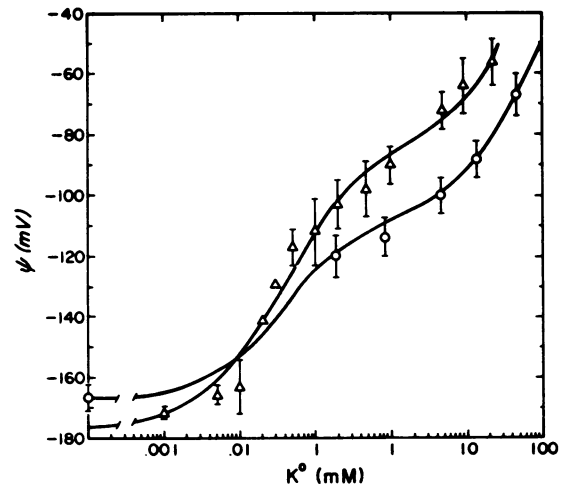

FIG. 4. K^+ dependence of total ψ in corn and barley roots as determined from the derived equations for ξ. (O): Experimentally obtained values in corn roots fitted with equation in text; (Δ): barley values reported by Mertz and Higinbotham (13) fitted with $\xi = \dfrac{(K^° + 0.0057)}{(K^° + 0.2185)} (0.00365 \ K^° + 0.0353)$ after concentration-to-activity correction for external KCl. For easiest visual comparison to reference 13 a semilog plot is used and standard deviations are shown.

The triangles represent the experimental values from Mertz and Higinbotham (13) and the corresponding calculated curve. Dr. Mertz furnished us with the primary data.

DISCUSSION

The description of the K^+ dependence of ψ_p rests heavily on two assumptions. First, uncoupling with FCCP is assumed not to alter any of the parameters of the Goldman-Hodgkin-Katz equation, thus allowing determination of the contribution of ψ_D to ψ. Although FCCP and other depolarizing agents might alter ionic permeabilities and activities, there is no firm evidence on this point. However, it is certain that FCCP is a powerful uncoupler of energy-linked transport, and in the absence of specific information on changes in permeability coefficients we feel justified in making the assumption that uncoupling affects only the energy-linked system.

The second assumption, that $\psi = \psi_D + \psi_p$, implies that the two components of ψ are potentials in series, whereas if the electrogenic system is in the plasmalemma (1, 6, 11, 17) they are more reasonably thought of as being in parallel (e.g. ref. 17). Circuits in parallel are unable to produce a potential which is the algebraic sum of the branches. Therefore, for the analysis we simply define the difference between ψ and ψ_D as ψ_p, the change in potential due to "pump" activity. Experimentally, the pump potential is the

difference in potential between coupled and uncoupled root cells: *i.e.* the potential that is lost when energy-linked transport is uncoupled.

So defined, the pump potential is an index of electrogenicity and an indicator of a close relationship between the electrogenic system and energy-linked transport. It is thus interesting that the minimum ψ_p in Figure 3 approximately coincides with the upper end of the Mechanism I range of ion absorption (2) and with the value of K° where K$^+$ influx becomes energetically downhill (about 0.4 mM). Also, ψ is least sensitive to changes in K° in the range of minimum ψ_p (Fig. 4).

The K° dependence of ψ_p (and ψ) in the Mechanism I range is qualitatively consistent with the chemiosmotic model previously proposed (6) in which the K$^+$ influx system is closely linked (possibly by the same carrier) with a H$^+$-pumping, electrogenic ATPase. Saturating the carrier with K$^+$, thus increasing the inward current of K$^+$, would have a depolarizing effect. At the higher K° values, the gradual rise in ψ_p is suggestive of K$^+$ stimulation of ATPase activity (9) and/or allosteric regulation of the K$^+$ carrier (4).

These comments only call attention to matters implicated in the relationship between ψ_p and K$^+$ influx. The more important point is that a relationship appears to exist which can be quantitatively explored with use of the technique described here for determining ψ_p. It is interesting that the analysis proves consistent with a common assumption about the putatively separate components of the cell potential; there is a diffusion potential which exists apart from the electrogenic potential, and which is unaffected by the operation of the electrogenic system and the energy-linked ion transport with which it is associated.

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