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

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ABSTRACT

The K⁺ dependence of normal (\(\psi\)) and diffusion (\(\psi_D\)) potentials in corn roots [Zea mays L., hybrid (A619 × Oh43) × A632] was determined experimentally and analyzed with respect to the parameter \(\xi\) [defined as \(\exp (F / RT)\)]. In the presence of 10 micromolar carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP), \(\psi\) behaved as 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 \(\psi_\circ\) and \(\psi_p\) (the deviation from \(\psi_\circ\) due to an electrogenic system), \(\psi\) was found to be a complex function of external potassium and to have a minimum value of 0.69 millimolar K⁺ activity outside the cell. Analysis of \(\psi\) allowed us to develop an equation which predicts a complicated K⁺ dependence of \(\psi\) 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, Mitchellean 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 (unipor); 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 \(\psi\) 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 systems.

We have found it useful to analyze \(\xi = \exp (F / RT)\). As a result we have been able to describe mathematically the behavior of \(\psi\) as a function of K⁺; to show that in the presence of 10 \(\mu\)M FCCP, \(\psi\) behaves as expected of a diffusion potential; and to characterize \(\psi_p\), the deviation of \(\psi\) from \(\psi_\circ\) 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): CaCl₂, 0.2; MES, 0.2; K₂SO₄, 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 K₂SO₄ concentration. Temperature during recordings was approximately 23 to 25 C. Recording circuitry was that described by Mertz and Amrten (12). Measured potentials were considered to exist entirely across the plasmalemma, i.e., with no potential difference across the tonoplast (14).
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 \( Cl^- \ll K^+ \) (about 1% in these experiments), it is also reasonable to omit the term \( pCI \ Cl^- \).

Equation 2 can therefore be simplified:

\[
\xi_D = \frac{1}{k^1} \cdot k^0 + \frac{pCI \ Cl^-}{k^1} \tag{3}
\]

In addition to its linearity with respect to \( K^+ \) this form is useful in that it allows one to estimate \( K^+ \) as the reciprocal of the slope and \(-pCI \ Cl^-\) as the \( x \) intercept.

For consideration of the electrogenic component, \( \psi_p \), and the total potential, \( \psi \), two further assumptions are required: (a) that the methods for revealing \( \psi_p \), e.g. application of FCCP, do not change \( K^+ \), \( Cl^- \) or \( pCI \); (b) that the total potential is functionally the algebraic sum of the diffusion potential and an electrogenic component, \( i.e. \):

\[ \psi = \psi_D + \psi_p \tag{4} \]

Then:

\[
\exp \left( \frac{F \psi}{RT} \right) = \exp \left( \frac{F \psi_D + F \psi_p}{RT} \right) \tag{5}
\]

or

\[
\xi = \xi_D + \xi_p = \frac{k^0 + pCI \ Cl^-}{k^1} \cdot \xi_D \left( \frac{k^0 + pCI \ Cl^-}{k^1} \right) \tag{6}
\]

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

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

We found one way to describe closely the deviation from linearity at low \( K^+ \) 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 \( \xi \) at \( K^+ = 0 \), 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 \( K^+ \):

\[
X = \frac{k^0 + A}{k^0 + A} + B \tag{7}
\]

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

\[
\frac{\xi \text{ observed}}{\xi \text{ predicted}} = \frac{k^0 + A}{k^0 + B} \tag{8}
\]

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

The experimentally determined values of \( \psi \) gave a linear regression for the higher values of \( K^+ \) (Fig. 2) which did not extrapolate to the same \( x \) intercept as that for \( \psi_p \) (Fig. 1). Based on the first of our assumptions (above), \( pCI \ Cl^- \) is to be considered constant under all experimental conditions, and thus despite the apparent linearity, \( \xi_p \) is also not constant in the range \( K^+ = 4.5 \) 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 \( \xi \). Together with the formula for \( \xi_D \), this allows us to calculate \( \xi_p \). The equations are rewritten in simple linear form:

\[
\xi_D = a_D (k^0 + \beta_D) \tag{9}
\]

\[
\xi = a (k^0 + \beta) \tag{10}
\]

where the \( a \) values are the slopes and \( a\beta \) values are the \( y \) intercepts. Thus in equation 9:

\[
a_D = \frac{1}{k^1}
\]

and

\[
\beta_D = pCI \ Cl^- \]

From this:

\[
\frac{\xi \text{ observed}}{\xi \text{ predicted}} = \frac{X \cdot a (k^0 + \beta)}{X \cdot a (k^0 + \beta)} \tag{11}
\]

\[
\xi_p = \frac{\xi \text{ observed} \cdot a (k^0 + \beta)}{\xi_D \cdot a_D (k^0 + \beta_D)} = \frac{k^0 + A}{k^0 + B} \cdot \frac{a (k^0 + \beta)}{a_D (k^0 + \beta_D)} \tag{12}
\]

**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 (\( K^+ = 0.19 \)).

**RESULTS.**

**Diffusion Potential.** Following introduction of 10 \( \mu \text{M} \) 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 \( \psi_p \) were reproducible to within 2 mv at a given \( K^+ \) in each experiment.

Figure 1a is a plot of \( \psi_D \) versus \( K^+ \). The corresponding plot of \( \psi_D \) 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.) \( \xi \) 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 \( \mu \text{M} \) FCCP; dotted line has slope of 58 mv/log unit. b: Corresponding plot of \( \xi_D \). Experimental points fall on the regression line \( \xi_D = 0.0303 K^+ + 0.0874 \). Means and standard errors are plotted.
from a diffusion potential. From the slope $K'$ is indicated to be 33 mm, very close to the value of 36 mm determined from tissue extracts. Extrapolation of the line through $\xi_0 = 0$ indicates that $p_{CI}Cl'$ 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 $p_{CI}Cl' = 0.17$. 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 $\psi$ 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 $\xi$ versus $K'$ in Figure 2.

At high $K'$, $\xi$ versus $K'$ is apparently linear. If $\psi_p$ were constant in this range the line should extrapolate to $-2.9$ mm, as with $\xi_D$ (Fig. 1b). That it extrapolates to $-10.2$ mm implies that either $p_{CI}Cl'$ is altered by FCCP, giving a false indication of the normal diffusion potential, or that $\psi_p$ 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 $p_{CI}Cl'$ is constant and that $\psi_p$ is not.

Electrogenic Potential. The electrogenic component, $\xi_e$, was determined as the ratio $\psi/\xi_D$ 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

$$\xi_e = \frac{K' + 0.0108}{K' + 0.1090} \cdot \frac{0.00132 (K' + 10.23)}{0.0303 (K' + 2.88)}$$

Converting this back to potential ($\psi_e = \frac{RT}{F} \ln \xi_e$) produces the curve for $\psi_e$ versus $K'$ (Fig. 3). At low $K'$ the first term of equation 12 ($\chi$) is dominant, producing a hyperbolic decline in $\psi_e$ with increasing $K'$. As $\chi \rightarrow 1$, the second term produces a slow rise in $\psi_e$. Only at very high $K'$ activity will $\xi_e$ (and $\psi_e$) 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 $\xi$ observed.

$$\xi = \frac{K' + 0.0108}{K' + 0.1090} \cdot \frac{0.00132 (K' + 10.23)}{0.0303 (K' + 2.88)}$$

**FIG. 2.** $\xi$-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 $\xi_D$ redrawn from Figure 1b.

**FIG. 3.** $K'$ dependence of electrionic component of cell potential computed from equations for diffusion and total potentials.

**FIG. 4.** $K'$ dependence of total $\psi$ in corn and barley roots as determined from the derived equations for $\xi$. (C): Experimentally obtained values in corn roots fitted with equation in text: (A): barley values reported by Mertz and Higinbotham (13) fitted with $\xi = \frac{K' + 0.0057}{(K' + 0.2185) (0.00365 K' + 0.0353)}$ 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 $\psi_p$ 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 $\psi_p$ to $\psi$. 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 $\psi$ 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 $\psi$ and $\psi_p$ as $\psi_e$, 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 \( \psi_p \) in Figure 3 approximately coincides with the upper 
end of the Mechanism I range of ion absorption (2) and with the 
value of \( K^\circ \) where \( K^+ \) influx becomes energetically downhill 
(about 0.4 mm). Also, \( \psi \) is least sensitive to changes in \( K^+ \) in the 
range of minimum \( \psi_p \) (Fig. 4).

The \( K^\circ \) dependence of \( \psi_p \) (and \( \psi \)) 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 \( K^\circ \) 
values, the gradual rise in \( \psi_p \) 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 \( \psi_p \) and \( K^+ \) influx. The more important point is 
that a relationship appears to exist which can be quantitatively 
explained with use of the technique described here for determining 
\( \psi_p \). 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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