A Quantitative Simulation Model for H\(^+\)-Amino Acid Cotransport To Interpret the Effects of Amino Acids on Membrane Potential and Extracellular pH\(^1\)

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ABSTRACT

The H\(^+\) cotransport of neutral and acidic amino acids induces transient depolarizations of oat coleoptile (Avena sativa L., var Victory) plasma membranes. The depolarizations, which are completed within 1 or 2 minutes, are followed by repolarizations that are nearly completed within another 2 or 3 minutes. Cysteine induced a two-phased alkalinization of the tissue free space during the electrical changes. The first phase was a rapid, linear increase in pH that coincided with the depolarization; the second phase was a slower, also linear, increase in pH that coincided with the repolarization. Reacidification did not occur until cysteine was withdrawn. Five other amino, basic, and neutral amino acids also induced persistent alkalinization of the free space.

The notable features of these measurements are that free-space pH was measured more directly than previously, that pH changes corresponded in time to the electrical potential changes, and that reacidification of the free space did not occur. The latter observation indicates that net H\(^+\) efflux did not occur during repolarization and that the repolarizing current was carried by some other ion. We propose that repolarization could have depended upon depolarization-induced changes in passive K\(^+\) fluxes combined with an enhanced H\(^+\) extrusion that increased until it equaled, but did not exceed, the enhanced influx of H\(^+\).

In support of the feasibility of our hypothesis, we present a quantitative simulation model for cotransport. The simulation model also provides an interpretation of the unique electrical effects of histidine and the basic amino acids. In addition, the model focuses attention upon the difficulties of interpreting H\(^+\)-anion cotransport.

Amino acids, sugars, and some other solutes appear to be taken into plant cells by cotransport with H\(^+\). Evidence for cotransport includes the solute-induced depolarizations of the cells membranes (4, 8, 18, 21, 24, 29, 30) and the solute-induced alkalinizations of the external medium (2, 3, 11, 13, 16, 18, 21, 23). The cotransported influx of H\(^+\) is considered to carry the depolarizing current, and for that reason the alkalinization of the tissue free space should parallel in time the depolarization of the cell membranes.

In most measurements of solute-induced pH changes in higher plants, macroscopic pH electrodes were used to measure the medium in which tissue sections were suspended. Thus, the pH changes in the tissue free space were measured only indirectly; diffusion through the tissue ensured that interior regions of the free space exchanged solutes slowly with the bulk medium. When cotransported solutes were added to the suspending medium, a gradual alkalinization proceeded usually for many minutes to several hours. Clearly the time course of such measurements bears no similarity to the electrical depolarizations that peak within 1 min.

The electrical depolarizations of the cell membranes were transient; that is, the membranes spontaneously repolarized in the continued presence of the solute; and within 5 min the \(E_m\) was generally stable once again. In the pH measurements just described, the medium occasionally reacidified, but again, very slowly. The usual interpretation for the \(E_m\) and pH reversals is that an enhanced H\(^+\) extrusion pump expels the excess H\(^+\) brought in by cotransport. This extrusion pump is probably a pH-sensitive ATPase (Ref. 9 and citations therein).

In our experiments we attempted to get better measurements of pH changes in the tissue free space. The result was that our solute-induced pH changes parallel in time the solute-induced electrical depolarizations. Unexpectedly, we observed no spontaneous reacidification of the free space. Thus, net H\(^+\) efflux was absent during repolarization, and the repolarizing current must have been carried by some other ion. Consequently, we propose that repolarization may depend upon depolarization-induced changes in passive K\(^+\) fluxes combined with an enhanced H\(^+\) extrusion that increases until it equals, but does not exceed, the enhanced influx of H\(^+\). Several authors have reported a K\(^+\) efflux that is concurrent with the solute-induced H\(^+\) influx (2, 3, 13, 16) and is considered a passive response to the electrical depolarization caused by the H\(^+\)-solute cotransport. This K\(^+\) efflux should offset some of the depolarization caused by the H\(^+\) influx, and the suggestion has been made that K\(^+\) efflux would function to reestablish the \(E_m\) (23). In support of the feasibility of our hypothesis, we present a quantitative simulation model for cotransport.

We also attempt to interpret, with the aid of the simulation model, the contrasting electrical effects of the neutral, basic, and acidic amino acids. Previous interpretations (8) of the electrical effects were the following: each neutral amino acid molecule enters the cell with one H\(^+\); each acidic amino acid anion enters

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\(^2\) Abbreviations: \(E_m\), electrical potential difference across a membrane; \(E_K\), Nernst potential for K\(^+\).
the cell with one H\(^+\) and one other, nonproton, cation; and each basic amino acid cation enters the cell by itself in a uniport. The cotransported H\(^+\) are eventually extruded from the cell, and the basic amino acid cations are eventually balanced by other ions. We reconsider these interpretations in the light of new data presented here and elsewhere and also offer an interpretation of the unique effects of histidine on the \(E_m\).

**MATERIALS AND METHODS**

\(E_m\) and pH measurements were made upon 4-d-old, dark-grown coleoptiles of *Avena sativa* L., var Victory that had been trimmed, put into special Lucite holders, and aerated for 3 h in a solution composed of 1 mM Ca(NO\(_3\))\(_2\), 1 mM NaCl, 1 mM KH\(_2\)PO\(_4\), and 0.25 mM MgSO\(_4\). This solution, designated here as 1X, was the basic medium for all experiments. Whenever other solutes were added, the pH was adjusted, when necessary, back to the original 5.0 or to other values with HCl or NaOH. Consequently, the Na\(^+\) or Cl\(^-\) occasionally varied from the initial 1 mM. A 10-fold concentration of 1X was used to cultivate the seedlings at 25°C, which was also the temperature for all other procedures.

After the aeration, a coleoptile, in its holder, was superfused with 1X during insertion of the glass microelectrode into an intact cell at the cut end. After a satisfactory microelectrode insertion and a steady \(E_m\) reading, the coleoptile could be superfused with different solutions by turning appropriate valves. The flow rate of 5 ml·min\(^{-1}\) was sufficient to saturate the rate of \(E_m\) change. Previous articles present further details of the electrophysiological techniques (4, 8, 9).

The coleoptiles were similarly superfused for the pH measurements. The pH semimicroelectrode (product No. MI-102; Microelectrodes, Inc., Londonderry, NH)\(^3\) was cone shaped at the end with the pH-sensitive tip about 50 \(\mu\)m long and 20 \(\mu\)m at the base. This electrode was inserted into one of the cut-open cells at the cut surface of the coleoptile. The electrode was pushed into the cell lumen until the increasing diameter of the nonsensitive glass shaft caused the electrode to plug the open end of the cut cell. The pH-sensitive portion of the electrode was thus wholly within the plugged, cut cell. Further insertion caused a deformation of the tissue and a sharp rise in pH, which until then had been about 5.0. The rise in pH depended upon the extent of the deformation. In general the electrodes were inserted just until deformation was observed. Within 5 to 20 min, the pH returned to 5.0 to 5.2 and stabilized. At that time, the coleoptile was superfused with 1X supplemented with a 4-mm concentration of one of the amino acids.

**RESULTS**

\(E_m\) Measurements. All tested amino acids induced depolarizations of oat coleoptile cell membranes (Figs. 1b, 2, and 3; Refs. 8 and 9, and unpublished data). The rate, extent, and duration of the depolarization phase varied among the amino acids, and the repolarization phase varied even more. The maximum depolarization was nearly always achieved within 2 min and often within 1 min. The depolarizations induced by the neutral and acidic amino acids were pH sensitive, and the spontaneous repolarizations were strong at all pH values tested. Glutamic acid induced large, spiky depolarizations, and occasionally two or three successive action potential-like depolarizations were triggered in the continued presence of this acid (data not shown).

\(^3\)Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.
wedged into one of the cut-open cells (Figs. 1a and 4). In the case of cysteine, the first phase consisted of a linear increase in pH of 0.1 to 0.3 units lasting for less than 1 min and coinciding in time with the electrical depolarization. The second phase was a slower linear rise in pH of 0.1 to 0.3 units lasting for 1 to 3 min and coinciding with the electrical repolarization. Within 2 to 4 min, the pH stabilized at an elevated value and remained there indefinitely unless the superfusion medium was switched back to 1X in which case the pH fell rapidly after an occasional transient rise. A transient fall in pH sometimes followed the addition of solute as well (see arginine tracing, Fig. 4).

**DISCUSSION**

Solute-induced depolarizations of the cell membrane are the consequence of inwardly directed electrical currents. In the case of H⁺ cotransport that current is carried by H⁺. Consequently, alkalinizations of the tissue free spaces should accompany the solute uptake and the Eₘ values change. Undelayed measurements of those alkalinizations cannot be obtained from the bulk media used to suspend tissue sections, and for that reason we embedded pH electrodes directly into the tissue. We observed two-phased, solute-induced alkalinizations. The first phase coincided with the solute-induced depolarization, and we consider both the pH and the Eₘ recordings to be undelayed measurements of cotransport events.

The spontaneous repolarizations following the depolarizations must come from outwardly directed electric currents. Most previous expositions of the cotransport hypothesis present only H⁺ as the current carrier in repolarization as well as in depolarization and imply that net H⁺ efflux would occur after sufficient acti-

![Fig. 2. Responses of the Eₘ to lysine at various pH values. The initial Eₘ values in mV are presented at the left.](image)

![Fig. 3. Responses of the Eₘ to histidine at various pH values. The initial Eₘ values in mV are presented at the left.](image)

vation of the H⁺ extrusion pump following the onset of cotransport. According to our observations, however, net efflux of H⁺ does not occur during repolarization. Therefore, the repolarizing current must be carried by another ion.

Under the conditions of our experiments, the repolarizing current may be carried by K⁺ moving passively in response to the reduced membrane polarity. The envisioned sequence is this: At the resting state, there is no net current across the membrane; that is, Eₘ is steady at -130 mV. Net flux of H⁺ is zero or nearly so since free-space pH is steady. When amino acids are applied, H⁺ influx increases and thus exceeds efflux. H⁺ extrusion becomes stimulated and increases until it equals, but does not exceed, the enhanced H⁺ influx. By the time net H⁺ flux is zero again the membrane has become repolarized, but some ion or ions other than H⁺ must have provided the necessary current. In the simplest case, K⁺, initially at passive equilibrium, carries the repolarizing current as it responds electrochemically to the reduced polarity of the membrane. When repolarization has been achieved, net K⁺ flux returns to zero. This case is a likely one because Eₓ is close to Eₘ at an external K⁺ concentration of 1 mM (1, 15, 20). In addition, K⁺ will respond electrochemically to Eₘ because of the relatively high permeability of the membrane to K⁺ and because of the high internal concentrations of K⁺ (1, 15, 20). Although the repolarizing current may be carried by passive ion fluxes, the active H⁺ extrusion pump is still essential for repolarization, and the ATP dependence of the spontaneous repolarization has been demonstrated (9). The enhanced activity of the extrusion pump slows and eventually halts net H⁺ influx.

The condition of initial passive equilibrium for K⁺ is not essential, however. Under certain conditions (e.g., higher or lower external K⁺ concentrations, Eₓ will not equal Eₘ and significant pumping will occur so that a pump and leak combination will exist. Still, an amino acid-induced depolarization will affect
A SIMULATION MODEL FOR COTRANSPORT

To examine the feasibility of our proposal for repolarization, we developed a quantitative simulation model for cotransport. The model embraces several conclusions and assumptions about cotransport that are summarized here: A saturable carrier system transports amino acids and H⁺ jointly across the plasma membrane driven by the additive electrochemical potential differences of the two solutes. For purposes of stoichiometry, we assume that one H⁺ protonates the carrier whether or not the amino acid itself is protonated, as in the case of the basic amino acids. Metabolic depletions or additions to the amino acid or H⁺ pools are disregarded for the first few minutes after the external addition of amino acids. At constant external amino acid concentrations, the bisubstrate carrier system is modeled to respond kinetically to H⁺ alone. Transport across the tonoplast is disregarded as is the contribution of the tonoplast to the $E_m$ and capacitance. The rate of H⁺ extrusion is taken to be controlled by cytoplasmic H⁺ concentration, and the cytoplasmic pH is assumed to change by no more than a few hundredths of a unit (28). We assume that the $E_m$ is influenced principally by four main fluxes: the net passive flux of K⁺, the amino acid cotransported influx of H⁺, other H⁺ influxes, and the H⁺ extrusion.

Equations for H⁺ and K⁺ Fluxes. In order to devise a simulation model for the pH and electrical effects of cotransport, it is necessary to obtain suitable expressions for the principal ion fluxes. The Goldman flux equation is generally considered to be appropriate for passive, independent ion fluxes not mediated by carriers (i.e. diffusion across the membrane) (17). Written for K⁺, the equation may take the form

$$J_K = P_{K}DG([K^+]_o - [K^+]_i)/[\theta]$$

where $P_{K}$ is the permeability coefficient, $DG$ is the electrochemical gradient, and $[\theta]$ is the concentration of the substrate. The Goldman equation can be used to describe the flux of K⁺ as a function of the electrochemical gradient and the permeability coefficient. However, it is important to note that the Goldman equation assumes that the ion flux is independent of the presence of other ions. In reality, the presence of other ions can affect the flux of K⁺, and this must be accounted for in a more detailed model of cotransport.

The addition of a new H⁺ influx begins, that associated with amino acid influx ($J_a$). This influx is in addition to all other H⁺ influxes ($J$) that occur prior to amino acid additions and go on subsequently. Cotransport kinetics may be described in terms of bisubstrate reaction kinetics, but these kinetics reduce to simple Michaelis-Menten kinetics when one of the substrates is held constant. In our model, the amino acid concentration is constant. Therefore, we have adopted the following equation to describe $J_a$.

$$J_a = J_{max}[H^+]/[H^+]_o + K_a$$

where $J_{max}$ is the maximum flux at a given amino acid concentration, and $K_a$ is a Michaelis constant. The absence of an electrical factor ($E_m$) is realistic according to the limited data available (5, 6) which indicate that cotransport is insensitive to $E_m$ at values appreciably above the reversal potential for cotransport.

We have chosen to model H⁺ extrusion ($J_e$) as though it were a first-order function of [H⁺]. Though the control of H⁺ extrusion by [H⁺], is uncertain, we have decided to test this simplest of hypotheses in our simulation model. Therefore, we have adopted the following equation

$$J_e = -k[H^+]_i$$

where $k$ is a rate constant, and outwardly directed cation fluxes are by convention negative.

There remains one additional flux for which we must write an expression. This is the H⁺ influx ($J_i$) that is independent of $J_e$ and largely masked by $J_a$. It is the return flux for $J_a$, and at the steady state prior to amino acid addition $J_i = -J_a$. That flux is quite problematical since it combines H⁺ diffusion and all cotransported influxes of H⁺ with mineral ions and organic solutes that may be taken back into the cell after leakage or other exudation. A direct measurement of $J_i$ is impossible because isotopic H⁺ quickly equilibrates with water. However, we can imagine that $J_i$ and $J_e$ may be considerable. For Neurospora...
mycelia at a resting $E_m$ of $-233$ mV, the leak current has been estimated at $6.7 \mu A \cdot cm^{-2}$ and the pump current at $8.0 \mu A \cdot cm^{-2}$ (26). The recycled current then is equivalent to 69 pmol cm$^{-2}$ s$^{-1}$. A large portion of this recycled current is probably carried by $H^+$. Since an expression for $J_r$ is open to much conjecture we simply set $J_r$ at a constant value equal to initial $-J_0$.

Three or 4 min after the addition of amino acid, when net $H^+$ flux is again zero, $J_1 + J_4 = k[H^+]_2$, where subscript 2 indicates final stable states. Prior to amino acid addition $J_1 = -J_0 = k[H^+]_1$, where subscript 1 indicates initial stable states. From these relationships, $\Delta pH_2 - \Delta pH_1 = \Delta pH_1 = -\log(1 + J_0/J_1)$, and it follows that if $J_0 = 10J_1$, then $\Delta pH_1 = -0.04$, which is about the detection limit for measurements of $\Delta pH_1$ (22, 28). This means that an amino acid uptake of 0.2 to 2 pmol cm$^{-2}$ s$^{-1}$ would produce a barely detectable $\Delta pH_1$ if the base-level $H^+$ influx and extrusion were 2 to 20 pmol cm$^{-2}$ s$^{-1}$. Even this $\Delta pH_1$ will sufficiently stimulate the $H^+$ extrusion pump to carry away the excess $H^+$ brought in by cotransport even though the pump is only a first-order function of $[H^+]_1$. The cotransport rate of 0.2 to 2 pmol cm$^{-2}$ s$^{-1}$ essentially covers the range of values for $J_{1,\text{max}}$ in higher plants (see citations in Ref. 7), and 2 to 20 pmol cm$^{-2}$ s$^{-1}$ is well below the estimated rate of $H^+$ cycling in *Neurospora*.

**Cotransport-Induced Changes in $[K^+]_o$, $E_m$, and $[H^+]_o$** Changes in $[K^+]_o$, $E_m$, and $[H^+]_o$ over successive, very short time intervals ($I < 1$ s) are computed from equations that incorporate fluxes that prevail at the start of each interval. The newly computed values for $[K^+]_0$, $E_m$, and $[H^+]_o$ are then incorporated back into the flux equations, where appropriate, to be used in the next round of computations. The equation for changes in $[K^+]_o$, over one of these intervals is

$$\Delta [K^+]_o = J_kI/V$$

(4)

where $V$ is the cytoplasmic volume. The equation for changes in $E_m$ is

$$\Delta E_m = (J_k + J_i + cI_A + J_e)IF/(AC)$$

(5)

where $F$ is the Faraday constant, $A$ the area of the cell surface, $C$ the specific capacitance, and $c$ the number of elementary charges that accompany each amino acid molecule. In the case of neutral amino acids the value is 1, but in the case of basic amino acids the number is 2. Anionic substrates such as acidic amino acids or mineral anions may have values of 1 or 2 for the negative charges are balanced by $H^+$ (or other cations) and 1 additional $H^+$ protonates the carrier molecule as well.

Changes in $[H^+]_o$ are computed from

$$\Delta [H^+]_o = [H^+]_{o,\text{initial}}(10^{(pH_3 + J_0)/(pH - 1) - 1})$$

(6)

where $[H^+]_{o,\text{initial}}$ is the concentration at the start of interval $I$, $B$ is the specific buffer capacity of the cytoplasm, and $p$ is the number of $H^+$ released into the cytoplasm per amino acid molecule. For neutral and basic amino acids, $p$ is 1, but for acidic amino acids, $p$ is 1 or 2.

In the case of histidine, the number of $H^+$ released per molecule depends on the pH of the cytoplasm, the extent of protonation of the transported molecule, and whether transport is cotransport or uniport. If we assume cotransport at all times (protonation of the carrier molecule irrespective of the protonation of the substrate molecule), then the number of $H^+$ released per histidine molecule is $c - 1 + K_3/[H^+] + [H^+]$. The value of $c$ depends on the fraction of histidine molecules that are protonated as they cross the membrane. If that fraction is equal to the fraction of molecules protonated in the external medium then $c = 1 + [H^+]_o/[H^+]_o + K_3$. $K_3$ is the acid dissociation constant of the side group and in the case of histidine is 10$^{-8}$ M.

**Assigned Values for the Simulation** In order to run the simulation program, initial values must be assigned to all the constants and variables. Most values are derived from measurements and their sources are cited; some other values are those that can be set experimentally (i.e. $[K^+]_o$ and $[H^+]_o$). Listed here are the values employed in what we call the standard run. $[K^+]_o$, 10$^{-3}$ M; $[K_+]_o$, 0.16 m M, 15, 20; $[H^+]_o$, 10$^{-3}$ M; $[H^+]_o$, 10$^{-10}$ M (14, 22, 27); $P_K$, 10$^{-4}$ cm s$^{-1}$ (15, 20); $E_m$, 0.13 V (9); $B$, 0.01 cm (22); C, 10$^{-2}$ F cm$^{-2}$ (17); $A$, 1.57 × 10$^{-4}$ cm$^{-2}$; $V$, 4.83 × 10$^{-12}$ L cm$^{-2}$ s$^{-1}$. $A$ and $V$ for parenchyma cells of oat coleoptiles were calculated from the dimensions of a cylinder 32 $\mu$m in diameter and 200 $\mu$m in length with $V$ equal to 3% of the total volume (15, 20). When $[H^+]_o$ is changed, $[H^+]_o$ also changes according to the relationship $pH_3 = 0.1 pH_3 + 6.5$ (14, 27).

Not all values have an origin wholly independent of the assumptions made in the model. In those cases, values were assigned that produced reasonable output by the model. In a sense, the model predicts the values that were assigned. Ultimately, independent measurements of those values must be obtained. $K_3$ was set equal to 10$^{-6}$ M in order for $H^+$ influx to nearly saturate at about $pH_3 = 5$ and nearly cease at $pH_3 = 8$ (see citations in Ref. 7). The value for $J_{1,\text{max}}$ was calculated from the observation that $J_{1,\text{max}}$ for amino acid uptake ranges from 1 to 10 pmol cm$^{-2}$ s$^{-1}$ (0.45 × 10$^{-16}$ to 4.5 × 10$^{-16}$ mol cell$^{-1}$ s$^{-1}$) to 2.1 pmol cm$^{-2}$ s$^{-1}$ (see citations in Ref. 7). For the first 2 min of the simulation, $J_{1,\text{max}}$ was assigned a value of zero. At the time of amino acid addition, $J_{1,\text{max}}$ gradually acquired a value of 10$^{-16}$ mol cell$^{-1}$ s$^{-1}$ with a half-time of 10 s. That was done to simulate the gradual exposure of the tissue to the added amino acid. When $J_{1,\text{max}}$ was assigned an instantaneous value of 10$^{-16}$, the cell depolarized at an excessive rate. The rate constant $k$ was assigned a value of 10$^{-8}$ L cell$^{-1}$ s$^{-1}$ in order for base-level $H^+$ extrusion (and negative $H^+$ influx) to equal $-10^{-16}$ mol cell$^{-1}$ s$^{-1}$.

**OUTPUT FROM THE SIMULATION MODEL**

Effects of Cotransport on $pH$ and $E_m$. Figure 5 presents the simulated effects of cotransport on $E_m$. For the neutral and basic amino acids and for histidine, the curves reasonably resemble their experimental counterparts. In particular, depolarization was greater at $pH_3$ 5 than at $pH_3$ 7; histidine was more sensitive to $pH_3$ than the other acids (8); spontaneous repolarizations were weak or absent in the basic amino acids; and the final $E_m$ in histidine depended upon the external $pH$. The model did not successfully simulate the effects of the acidic amino acids if it is assumed that the anionic molecule entered the cell with two $H^+$. The curve for neutral amino acids at $pH_3$ 5 is the standard run. The legend for Figure 5 presents the changes in the initial values of the simulation model required to generate the other curves.

Figure 6 presents the simulated effects of neutral amino acids on cytoplasmic $pH$. The effect of cotransport on $pH$ can be inferred from measurements of free-space $pH$ (Figs. 1a and 4). For oat coleoptiles, there is an alkalization of the free space that persists until the solute is withdrawn from the medium. Presumably there is an inverse reaction within the cell, that is, an acidification that does not reverse itself. Figure 6 illustrates that the cotransport model simulates the expected results. Note that the very small magnitude of cytoplasmic $pH$ change that occurs if $J_0$ and $-J_r$ are assumed to be large with respect to $J_r$, despite the assumption of only first-order dependence of $J_r$ on $[H^+]_o$. With present measurement techniques, such changes could hardly be detected (22, 28). If $H^+$ recycling is low, the $pH_3$...
Fig. 5. Simulated responses of the $E_m$ to amino acids. The simulated additions of the solutes were made at time zero. The upper and lower curves are responses at external pH values of 5 and 7, respectively. For the neutral amino acids, the simulation program was the standard run (except for changes in the external pH) described in the text. For the basic amino acids, $c$ and $p$ (from equations 5 and 6) were assigned 2 and 1, respectively. For histidine, $c'$ and $p'$ were computed from pH_{in} and K_{a'} (=10^{-6} M)$ (see text). For the acidic amino acids, $c = 1$ and $p = 2$.

Declines further, and repolarization is sluggish.

**fluxes.** Figure 7 illustrates that the simulation model generates fluxes compatible with observation or expectation. The amino acid-cotransported H\(^+\) influx ($J_a$) is offset by increases in H\(^+\) extrusion ($J_e$), but the net H\(^+\) flux ($J_a + J_e + J_{c\text{s}}$) never assumes negative values (i.e. never becomes outwardly directed). Net K\(^+\) flux ($J_k$) mirrors the $E_m$, and like the net H\(^+\) flux, rises and falls quickly, quite unlike the long-term, solute-induced K\(^-\) effluxes measured previously from bulk preparations (2, 3, 13, 16). The net flux of cations into the cell ($J_a + J_e + J_{c\text{s}}$) is a difference curve for net H\(^+\) influx and net K\(^+\) efflux. It is also related to the first derivative of the corresponding $E_m$ tracing in Figure 5a. In our model, both H\(^+\) extrusion and passive K\(^+\) fluxes are essential to repolarization. Elimination of either leads to permanent depolarizations in the presence of amino acids. (For simulations, $P_k$ or $k$ was set to zero; output is not presented. See Reference 9 for experimental evidence for the essentiality of H\(^+\) extrusion).

**Basic Amino Acids.** The standard run of the simulation model assumes a neutral and uncharged amino acid cosubstrate. For the basic amino acids, we assumed a positively charged cosubstrate plus a cotransported H\(^+\). Thus, the ratio of H\(^+\) released to charges delivered is 1:2. Consequently, the H\(^+\) extrusion pump can carry off only half the delivered charge at steady pH values, and the remainder is carried off by K\(^+\) responding to the reduced polarity. After the return of steady $E_m$ and pH_{in}, $J_k = J_{c\text{s}}$. The effect is a simulated depolarization that recovers weakly or not at all (Fig. 5b) and corresponds with observed phenomena (Fig. 2; Ref. 8).

The simulation model is also compatible with the uniprot hypothesis for the transport of the basic amino acids. (Let $c = 1$ and $p = 0$ in the simulation.) Amino acid uptake becomes a cation influx with no accompanying H\(^+\) influx. The H\(^+\) extrusion...
pump is not stimulated and repolarization does not occur. Consequently, the model does not discriminate between the uniport and cotransport hypotheses, but it does show that the uniport hypothesis (8) is not essential in order to interpret the electrical data. Resolution therefore depends upon other evidence. Recent arguments for the cotransport (23), uniport (12), and antiport (19) of basic amino acids have been presented, and perhaps the transport stoichiometry for H⁺ varies among tissues or even within a tissue. The weak alkalinizations of the free space in our experiments (Fig. 4) may or may not signify cotransport (see Ref. 12). A quantitative analysis of published transport experiments led to the conclusion that amino acids are transported via two channels. One is a general channel that transports all the amino acids (including the basic). The second channel has high affinities only for the basic amino acids (7). An attractive, but unsupported hypothesis is that the first is a cotransport channel and the second a uniport channel.

Histidine. To simulate histidine cotransport, $K_e$ was set to $10^{-6}$ M, and the pH-dependent repolarization (Fig. 5c) resembled observed responses (Fig. 3). The explanation is that lower external pH values induce lower pH values and higher rates of uptake of H⁺ from both $J_e$ and $J_r$. The cytoplasmic pH stabilizes at 6.93 at an external pH of 5, and a portion of the histidine molecules brought into the cytoplasm remains protonated. These protonated histidines are similar in effect to the basic amino acids and must be offset by K⁺ efflux. At higher external pH values, the cytoplasmic pH is higher, and uptake rates of H⁺ are not as great. The pH, stabilizes at 7.18 at an external pH of 7, and the fraction of protonated histidine molecules is smaller. After the return of steady $E_n$ and pH, (no net H⁺ flux), K⁺ efflux is a function of $J_e$ and the fraction of histidine molecules that accumulate in the protonated form. Therefore $-J_K = J_e[H^+]/([H^+] + K_e)$. Both $J_e$ and [H⁺] are increased at lower pH, values, and $E_n$, which is related to $J_K$ by equation 1, is less negative at low pH. The value of c (whether 1 or 2) has less than a 2 mV effect on final $E_n$, but the effect on the peak depolarization is considerable. If we assume that $c = 1 + [H^+]/(K_e[H^+])$, c could account for the high sensitivity of histidine-induced depolarizations to pH (Ref. 8; Fig. 5c).

Acidic Amino Acids. The simulation model does not work for acidic amino acids unless we modify the model or assume the participation of nonproton cations in the cotransport. If the carrier is assumed to transport an anionic amino acid, a H⁺, and another cation, then the simulation is indistinguishable from that for neutral amino acids (as are experimentally observed aspartic acid-induced depolarizations [8]). If the carrier transports an anionic amino acid and one H⁺, the uptake is electrogenic, contrary to observation. If the carrier transports an anionic amino acid and two H⁺, the simulated cell undergoes a transient depolarization followed by a large hypolarization as a consequence of cytoplasmic acidification, since the H⁺ to charge ratio would be 2:1 (Fig. 5d). Nevertheless, several authors have con-
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cluded that various anions are cotransported with more than one H\(^+\) (23, 24, 29, 30).

CRITIQUE OF THE MODEL AND CONCLUSIONS

According to the simulation model, a first-order substrate control of the H\(^+\) extrusion pump is sufficient to maintain \(E_{m}\) and cytoplasmic pH against the influx of amino acid-cotransported H\(^+\). However, adequate control requires a base-level extrusion activity greater than the rate of cotransported influx. Higher-order substrate control, or controls by other factors, may allow lower base-level rates of extrusion. Because the decline in cytoplasmic pH may be very small, measurements of cytoplasmic pH at present levels of resolution may be misleading regarding the adequacy of [H\(^+\)], for control of the H\(^+\) extrusion pump.

The simulation model is extremely simple, composed, as it is, of four primary flux equations and three principal cellular variables (\(E_{m}, [H^+]\), and [K\(^+\)]). The flux equations in particular are simplifications. It is well known that the kinetic constants for solute fluxes are, in fact, not constants but that the apparent \(K_{m}\) and \(V_{max}\) values are significantly affected by changes in cytoplasmic pH, for example, in the case of H\(^+\) cotransport (24, 25). We have tested modifications of the Michaelis-Menten equation, but the changes in the simulation output did not particularly bear upon our principal objective of interpreting the repolarization. The model was kept simple in order to focus attention upon the function of essential components of the model, unobscured by unnecessary details, and because there is insufficient information for further refinement in most cases.

Nevertheless, additional features of cellular physiology may be incorporated into the model. The resting \(E_{m}\) may have a component dependent upon the extrusion of H\(^+\) arising from metabolic sources, or upon other electrogenic activities. If \(E_{m} \neq E_{K}\), appropriate uptake or extrusion pumps may be incorporated into the model in order to achieve an initial steady state. The model still produces satisfactory amino acid-induced depolarizations that depend upon electropheretic K\(^+\) fluxes.

The simulation model has performed its intended function of demonstrating the feasibility of our hypothesis that the spontaneous repolarization need not be the consequence of net H\(^+\) extrusion which, according to our data, does not occur during repolarization. Instead, the model demonstrates the feasibility of the hypothesis that repolarization depends upon depolarization-induced changes in passive K\(^+\) fluxes combined with an enhanced H\(^+\) extrusion that increases until it equals, but does not exceed, the enhanced influx of H\(^+\).

The model has also assisted us in a reexamination of our previous interpretation of basic amino acid uptake (8). The weak free-space alkalization can be taken to indicate at least some cotransport of H\(^+\) with the basic amino acids, and the simulation model demonstrates that a H\(^+\) cotransport of basic amino acids could generate the observed, unreversing depolarizations. The model also suggests an interpretation for the pH-dependent effects of histidine on repolarization and peak depolarization. The unaltered model cannot accommodate the cotransport of acidic amino acids, or other anions, with two H\(^+\). Some modification of the model required is if the cell is to avoid hyperpolarization in the face of a 2:1 delivery of H\(^+\) and unit electrical charges.

LITERATURE CITED


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