Electrical Evidence for Different Mechanisms of Uptake for Basic, Neutral, and Acidic Amino Acids in Oat Coleoptiles[1,2]

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ABSTRACT

The application of neutral or acidic amino acids to oat coleptiles induced transient depolarizations of the membrane potentials. The depolarizations are considered to reflect H^+-amino acid co-transport, and the spontaneous repolarizations are believed to be caused by subsequent electrogenic H^+ extrusion. The basic amino acids depolarized the cell membrane strongly, but the repolarizations were weak or absent. The depolarizations induced by the basic amino acids were weakly sensitive to manipulations of the extracellular and intracellular pH. The depolarizations induced by the other amino acids, in contrast, were more strongly affected by the pH changes. Several amino acids induced distinct but diminished depolarizations in the presence of 2,4-dinitrophenol or cyanide, but the repolarizations were generally eliminated. These experiments support the co-transport theory but suggest somewhat different mechanisms for the transport of the neutral, acidic, and basic amino acids. We suggest that the neutral amino acids are co-transported with a single H^+ and that accumulation depends upon both the ΔpH and the membrane potential components of the proton motive force. The acidic amino acids appear to be accumulated by a similar mechanism except that the transport of each molecule may be associated with a cation in addition to a single proton. The permanently protonated basic amino acids appear not to be co-transported with an additional proton. Accumulation would depend only on the membrane potential component of the proton motive force.

Application of sugar or amino acid solutes to a variety of plant tissues induces partial and transient depolarizations of the cell membrane potentials (2, 3, 8, 12, 17, 20, 22). These depolarizations were generally considered by the authors to reflect carrier-mediated co-transport of the solutes with H^+ across the membrane. The basic features of the co-transport hypothesis are the following: An electrochemical potential difference in H^+ is maintained across the cell membrane by active H^+ extrusion. This extrusion probably employs a membrane ATPase (9, 19, 21). The electrochemical potential difference in H^+ or the proton motive force (pmf $= 59\Delta pH - \Delta E$; units in mv, T = 25 C) drives the accumulation of solutes. (Actually, the driving force on the solute should also take into account the concentration difference of the solute itself.) Details of the carrier system are not known, except that solute inducible protein molecules appear to be employed, at least in several eucaryotic and procaryotic microorganisms (12, 20, 25). According to the mobile-carrier hypothesis the H^+ and the solute molecule both associate with the carrier at the outer surface of the membrane. This charged ternary complex then moves across the membrane in response to the electric potential gradient. At the inner surface the complex dissociates. A pH difference across the membrane enhances uptake by promoting formation of the ternary complex at the outer surface and dissociation of the complex at the inner surface.

The evidence in favor of H^+ co-transport with sugar and amino acid solutes is as follows: (a) solute uptake is enhanced at lower external pH values (3, 6, 18); (b) solutes induce transient depolarizations of cell membranes (see above); (c) sugar solutes induce transient basifications of the external medium (7, 11, 17, 20, 24) and (d) fusicoccin and IAA, compounds that stimulate H^+ extrusion, promote solute uptake (1, 3, 5, 18). The solute-induced depolarizations and the solute transport appear to be closely linked, although both types of measurements have seldom been made in the same investigation. Both respond in parallel to external concentrations of solutes (2, 3, 6, 13, 16, 17, 20), the external pH (2, 3, 6, 13, 17, 22), the presence of inhibitors (3-6, 13, 18), the presence of competing solutes (6, 18; Kinraide, unpublished results), the aging of wounded tissue (18), and, perhaps most tellingly, induction for solute uptake (12).

Within 2-5 min after the introduction of the solute the membrane recovers most of the polarity initially lost. This repolarization is considered to reflect the electrogenic extrusion of the excess H^+ introduced during co-transport (16, 24). Eventually electrical stability is achieved, presumably when the H^+ efflux pump has adjusted to the additional burden imposed by the co-transported influx. If the solute is suddenly withdrawn a transient hyperpolarization is generally seen (2, 3, 16). This could be interpreted as the continued hyperactivity of the H^+ extrusion pump and perhaps as a reverse co-transport resulting from the solute gradient. The model predicts a transient basification of the external medium parallel in time with the depolarization of the cell membrane. In a few cases such transient changes in pH have been measured as noted above. In addition, glucose and 3-O-methyl glucose caused transient reversals of fusicoccin-induced H^+ extrusion from maize root sections (1).

Our experiments provide additional tests of the H^+ co-transport theory for acidic, basic, and neutral amino acids. We measured cell membrane potentials in oat coleoptiles in response to the application of amino acids after the manipulation of the intracellular and extracellular pH and in the presence of metabolic inhibitors. The results support the theory of H^+-amino acid co-transport associated with active, electrogenic H^+ extrusion for the acidic and neutral amino acids but not the basic acids. We have presented a preliminary report of our results elsewhere (10).

MATERIALS AND METHODS

Our procedures have been reported in detail elsewhere (3). In brief, 4-day-old, dark-grown coleoptiles 15-25 mm in length were trimmed and aerated for 3-7 h in a solution composed of the following in mM concentrations: 1 Ca(NO₃)₂, 1 NaCl, 1 KH₂PO₄, and 0.25 MgSO₄ (pH 5.0). This medium, designated here as 1×,

Table I. *Survey of Amino Acid-induced Electrical Effects*

The depolarizations of the cell membrane by the addition of 4 mM concentrations of the amino acids and the spontaneous repolarizations were measured at pH 5.0 in 1X medium at 25 C. pI is the isoelectric point of the amino acid. Na^+ and Cl^- concentrations are the concentrations of the medium after the addition of the pH-adjusted amino acid-containing solution.

Amino Acid	pI	Depolarization	Repolarization within 5 min	Na^+ Concentration	Cl^- Concentration
		mv ± SE		*mM*	
Asp	2.87	16.9 ± 1.5 (7)[a]	12.4 ± 2.1 (7)	4.7	1.0
Glu	3.22	78.4 ± 3.7 (9)	68.0 ± 5.6 (8)	4.4	1.0
Cys	5.02	60.6 ± 8.1 (5)	47.0 ± 9.0 (5)	1.0	1.0
Gln	5.65	23.7 ± 0.7 (3)	16.7 ± 0.9 (3)	1.0	1.0
Met	5.75	14.3 ± 1.9 (3)	11.0 ± 3.1 (3)	1.0	1.0
Trp	5.88	8.3 ± 1.0 (4)	5.8 ± 1.0 (4)	1.0	1.0
Ala	6.02	27.3 ± 1.2 (3)	19.3 ± 0.9 (3)	1.0	1.0
Thr	6.53	28.3 ± 2.7 (4)	16.3 ± 2.4 (4)	1.0	1.0
His	7.58	40.7 ± 4.8 (7)	11.5 ± 3.1 (6)	1.4	5.0
Orn	9.71	48.3 ± 4.8 (3)	3.7 ± 1.2 (3)	1.0	5.0
Lys	9.74	49.8 ± 1.8 (4)	10.0 ± 2.5 (4)	1.0	5.0
Arg	10.76	31.4 ± 1.5 (5)	1.2 ± 0.8 (5)	1.0	5.0
AIB		17.0 ± 0.8 (7)	12.9 ± 1.8 (7)	1.0	1.0

[a] Number of observations.

differs from the 1X used before mainly in pH. A tenfold concentration of the medium was used to cultivate the seedlings at 25 C, which was also the temperature for all other procedures. The tissues were perfused with 1X at the time the cells were impaled with glass capillary microelectrodes. Subsequent to a satisfactory electrode emplacement (*i.e.* change in potential difference < 1 mv/min, noise level < 1 mv, and tip resistance < 100 megohms, in most cases) a valve was turned to admit a solution at a rate of 3 ml/min. This solution was generally 1X plus an amino acid or some other solute. Sometimes the solution was changed several times, the potential difference being recorded continuously.

Except where noted the pH was always 5.0; adjustments were always made with minimal amounts of NaOH or HCl which then caused changes in Na^+ and Cl^- concentrations. Specifically: adjustment of 1X to pH 7.0 elevated the Na^+ concentration to 1.4 mM; addition of 1 mM HXA[3] to 1X and adjustment to 5.0 elevated Na^+ and Cl^- to 1.1 and 2.0 mM, respectively; adjustments of 4 mM acetic acid or 0.1 mM DNP in 1X to 5.0 elevated Na^+ to 3.6 or 1.1 mM, respectively; and adjustment of 4 mM IMD or 1 mM KCN in 1X to pH 5.0 elevated Cl^- to 5.0 or 2.0 mM, respectively. The changes in Na^+ and Cl^- resulting from titrations of the amino acids are listed in Table I.

RESULTS

Survey of Amino Acids. Different amino acids had different effects on the membrane potential (Table I). Glutamic acid and cysteine induced strong, rapid, and transient depolarizations when applied as 4 mM solutions in 1X at pH 5.0. The other acidic and neutral amino acids caused weaker and slower depolarizations and repolarizations (Fig. 1a). The extent of the repolarization for nine acidic and neutral amino acids was fairly constant at 73%. Only glutamic acid and threonine repolarizations departed considerably from that mean (Fig. 2). Repolarization in the strongly basic amino acids (ornithine, lysine, and arginine) was very weak

[3] Abbreviations: DNP: 2,4-dinitrophenol; HXA: hydroxylamine·HCl; IMD: imidazole; pI: isoelectric point; AIB; α-aminoisobutyric acid.
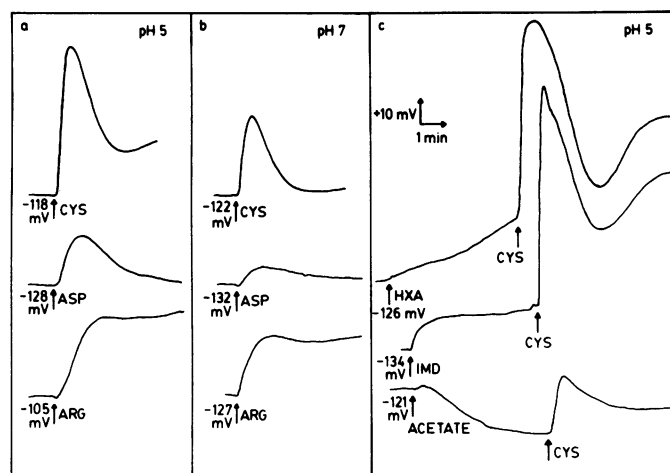

FIG. 1. Sample tracings of chart records showing the response of the membrane potential differences to amino acids in various solutions. Solutes were added to 1X (at pH 5.0 or 7.0) at the times indicated by the arrows. Once a solute was added it remained in the solution. The concentration of IMD and the amino acids was 4 mM; HXA was 1 mM. A potential difference is given at one of the arrows for each tracing.
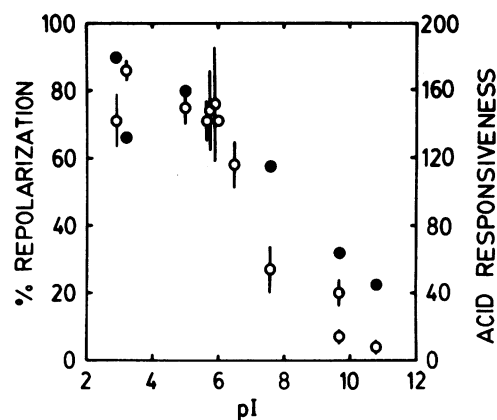

FIG. 2. Per cent repolarization (O) and acid responsiveness (●) in the presence of amino acid solutes as a function of their pI. The conditions for the per cent repolarization were as described in Table I, and the points may be identified by reference to that table. A value for AIB, which repolarized at 75% ± 10 (7), is not plotted. The values for acid responsiveness are equal to the sums of the vertical bars for each amino acid in Figure 3. The value for AIB is 222. Circles indicate means and bars the SE.

(Fig. 1a), and in the weakly basic amino acid histidine, the repolarization was moderate. See Figures 1 and 2 and Table I. Glucose and sucrose also induced transient depolarizations, but glucosamine and glucuronic acid affected the potential difference only weakly, preventing their use in a comparative study on the effects of charged sugar derivatives on the membrane potentials.

The initial cell membrane potentials were quite uniform with a mean value of −124 ± 10 mv (SD). Potentials above −100 mv or below −150 mv were practically never encountered. The variation in the initial membrane potential had no observed effect upon the subsequent amino acid-induced depolarizations. Likewise, the external tip potentials of the electrodes (14 ± 8 mv, mean and SD) had no significant effect upon the initial cell membrane potentials.

Adjustments of Intracellular and Extracellular pH. The differential repolarizations among the amino acids inspired additional experiments to compare the electrical effects of the acidic, basic, and neutral amino acids. The first experiment was to test the effect of the amino acid on the membrane potential at the more alkaline pH of 7.0. Prior observations showed both the depolarization and

the rate of uptake to be diminished for most amino acids at higher pH values. The inhibition of depolarization ranged from 30 to 61% for aspartic acid, glutamic acid, cysteine, AIB, and histidine. The strongly basic acids lysine and arginine depolarized the membrane at pH 7.0 only 15 and 26% less, respectively, than at pH 5.0. See Figures 1 and 3 and Table II.

We observed earlier that a brief preincubation of the coleoptile sections in NH_4Cl, IMD, or HXA in 1X at pH 5.0 had a stimulatory effect upon the depolarization induced by 4 mM AIB. Acetate, on the other hand, had an inhibitory effect. Weak acids that can penetrate the cell membrane as unionized molecules have an acidifying and hyperpolarizing effect after intracellular ionization; weak bases do the opposite (9, 14, 23). Consequently, we tested
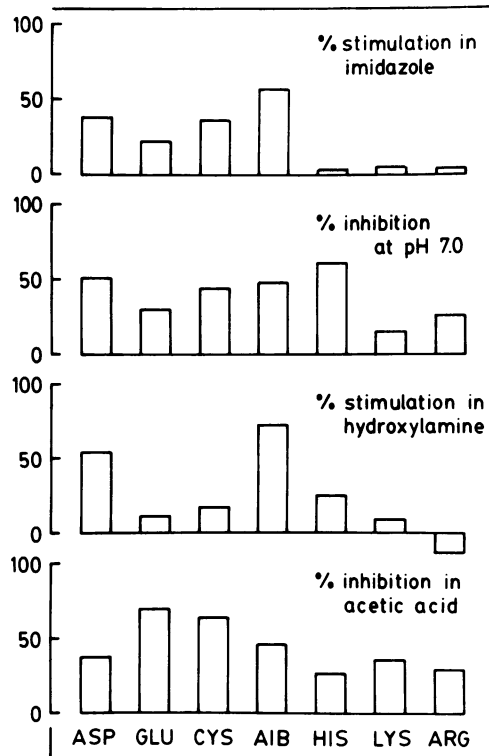

FIG. 3. Effect of pH manipulations on the amino acid-induced depolarizations of the membrane potential. Conditions were as described in Table II.

Table II. *Influence of pH on Amino Acid-induced Depolarizations*

The depolarizations of the cell membrane induced by the addition of 4-mM concentrations of the amino acids were measured at pH 5.0, at pH 7.0, and in the presence of 4 mM IMD, 1 mM HXA, or 4 mM acetic acid. Prior to the addition of the amino acids the tissues were perfused for 2 min in 1X at pH 7.0 or for 5 min with 1X supplemented with one of the other solutes at pH 5.0. The amino acids were added in the continued presence of the pretreatment solutes.

Amino Acid	IMD	pH 7	HXA	Acetic Acid
		mv ± SE		
Asp	23.3 ± 3.2 (3)[a]	8.2 ± 0.8 (4)	26.0 ± 2.6 (4)	10.7 ± 0.9 (3)
Glu	95.3 ± 0.9 (3)	54.7 ± 7.7 (3)	87.3 ± 0.3 (3)	24.3 ± 4.7 (3)
Cys	82.3 ± 0.9 (3)	34.2 ± 2.0 (6)	71.0 ± 2.5 (4)	22.3 ± 0.3 (3)
AIB	26.8 ± 3.1 (4)	8.8 ± 0.2 (4)	29.3 ± 3.6 (6)	9.3 ± 0.9 (3)
His	42.0 ± 1.5 (3)	15.7 ± 1.6 (7)	50.7 ± 1.8 (3)	30.0 ± 2.3 (3)
Lys	52.5 ± 3.5 (4)	42.2 ± 3.2 (5)	54.3 ± 6.5 (3)	32.3 ± 1.8 (3)
Arg	32.3 ± 2.3 (3)	23.1 ± 2.2 (9)	27.3 ± 1.2 (3)	22.3 ± 1.7 (3)

[a] Number of observations.

the effects of a 5-min preincubation in 4 mM IMD or 1 mM HXA or 4 mM acetic acid upon the depolarizations induced by the seven amino acids mentioned above. The addition of imidazole to the 1X medium induced a relatively rapid depolarization of the membrane that stabilized for a few minutes at an average of 12 mv (Fig. 1c). (After about 4 min the depolarization resumed.) After 5 min a 4 mM concentration of amino acid was added (imidazole still present). The ensuing depolarizations were enhanced by 22–57% for the acidic and neutral amino acids, but the effect on the depolarizations induced by the basic amino acids was no greater than 5% (Fig. 3 and Table II). The addition of HXA to the 1X medium initiated a slow depolarization of the membrane averaging 16 mv after 5 min (Fig. 1c). HXA strongly stimulated the depolarizations induced by aspartic acid and AIB but had smaller effects otherwise. Acetic acid had the effect of hyperpolarizing the membrane by an average of 18 mv (Fig. 1c). Amino acid-induced depolarizations were depressed in acetic acid, but to a lesser extent for histidine, lysine, and arginine. See Figures 1 and 3 and Table II.

An index of acid responsiveness of the cell to each of the seven amino acids tested was computed by adding vertically the bars in Figure 3 for each acid. The results are plotted in Figure 2. The correlation coefficient between per cent repolarization and index of acid responsiveness is 0.837 for seven amino acids ($P < 0.05$). Since the pI of AIB is not precisely known the values for that acid have not been plotted in Figure 2.

Effects of Cyanide and DNP. A series of experiments was done with metabolic inhibitors in order to gain further insight into the nature of the spontaneous repolarization. The addition of 0.1 mM DNP in 1X caused a gradual depolarization of the membrane to about −40 mv after about 15 min. The addition of 1 mM cyanide induced a rapid depolarization to about −20 mv in about 2 min. This was followed by a repolarization to about −80 mv after an additional 10 min. The potential difference finally stabilized at about −50 mv after about 10 min more. The addition of 4 mM aspartic acid, glutamic acid, cysteine, lysine, or arginine to inhibitor-treated cells induced distinct but diminished depolarizations. AIB, a weak depolarizer under ordinary conditions, produced no significant depolarizations. More significantly, the repolarizations were eliminated completely or significantly reduced except that glutamic acid generally induced transient depolarizations upon initial exposure; subsequent depolarizations were like those for the other acids. See Figure 4 for sample traces. The withdrawal of amino acids from inhibitor-treated cells induced small but distinct repolarizations (Fig. 4, traces 1 and 5).

DISCUSSION

Our initial observations on spontaneous repolarizations (Fig. 2) led us to propose the models for transport presented in Figure 5 and described below. The models predict a minimal effect of ΔpH on depolarizations induced by basic amino acids; the depolarizations induced by them were indeed weakly responsive to pH manipulations. We consider this to be good evidence that the co-transport of the strongly basic amino acids with H^+ is weak or absent. Instead, the data are consistent with the facilitated diffusion of a permanently charged cation whose electrochemical potential gradient itself generates the driving force. Accumulation ratios could approach about 100. Stability of the membrane potential difference could be achieved at a new and lower level by the stoichiometric influx of anions or efflux of cations other than H^+ in response, perhaps, to the altered potential difference (Fig. 5).

The acidic and neutral amino acids induced electrically similar responses. The acidic amino acids induced a depolarization of the membrane and thus must have entered the cell as a positively charged complex composed of two cations accompanying each amino acid anion. In no case did the membrane hyperpolarize in
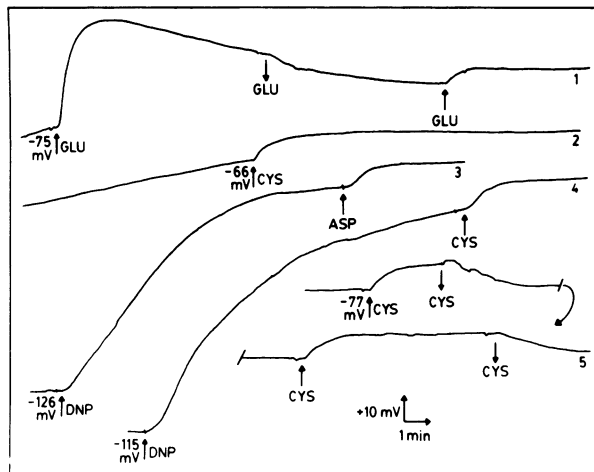
FIG. 4. Sample tracings of chart records showing the response of the membrane potential differences to amino acids in DNP and cyanide. An upward-pointing arrow indicates the addition of a solute, and a downwardly pointing arrow indicates the removal of a solute. The concentration of the amino acids was 4 mM; of DNP, 0.1 mM; and of KCN, 1 mM. At the time of the first arrows in traces No. 1 and 2 the tissues had been exposed to KCN for 15 and 35 min, respectively. At the time of the first arrow in trace No. 5 the tissue had been exposed to DNP in 0.1× for 22 min.
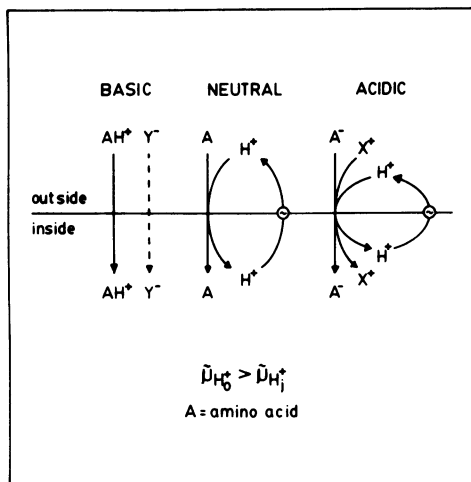

FIG. 5. Models proposed for the transport of basic, netural, and acidic amino acids across the cell membranes of oat coleoptiles. The symbol μ_{H^+} stands for the electrochemical potential for hydrogen ion. Further explanation is provided under "Discussion."

the presence of an amino acid, indicating that only one H^+ was extruded per charge delivered into the cell. A possible explanation for the transport of the acidic amino acids is a three-way co-transport of an anionic amino acid, a H^+, and some other cation which accumulates as counterion to the acidic anion (Fig. 5). We reason that the acidic anions are not cotransported with two H^+ since the extrusion of both would hyperpolarize the cell but failure to extrude both would lead to a progressive acidification of the cell. In addition, co-transport with two H^+ should make the depolarization hypersensitive to ΔpH, but the acid indexes for aspartic acid and glutamic acids considered together are not higher than those for cysteine and AIB.

The depolarizations induced by glutamic acid and cysteine in the presence of HXA and IMD were less stimulated than the depolarizations induced by aspartic acid and AIB. The explanation may be that depolarizations for the former acids were already very high and that further stimulation became more difficult as

the potential difference approaches or rises above the diffusion potential of −40 to −50 mv. In addition, the carrier sites may have been nearly saturated, making stimulation of uptake more difficult, at least on a percentage basis. Glutamic acid may be anomalous in some ways. Depolarization of the membrane as a function of concentration stepped up suddenly at 0.2 mM (22); a single application of glutamic acid occasionally induced repeated depolarizations of the membrane (Stebbins and Kinraide, unreported results); and the membrane occasionally repolarized after the addition of glutamic acid in the presence of inhibitors (Fig. 4, trace 1).

Previous investigators (15) concluded that the anionic, acidic amino acids enter *Staphylococcus aureus* cells with a single H^+, and they considered the transported complex to be neutral and responsive only to ΔpH and not to the potential difference. These authors explicitly described glutamate and aspartate uptake as electroneutral. If this were the case in our tissues, the initial depolarizations would be difficult to explain. In addition, one would expect an acidification of the cell and a hyperpolarization as the H^+ is extruded, as occurs when tissues are exposed to weak acids (14, 23; Fig. 1c). These investigators, who did not have the benefit of electrical measurements, proposed a uniport for the cationic basic amino acids. Our electrical data strengthen that proposal.

Research on amino acid transport in higher plants remains in a rather undeveloped state in that coordinated measurements of electrical phenomena, amino acid and mineral ion fluxes, and cytoplasmic pH have not been done in detail. It is entirely possible that plants may simultaneously employ proton motive force-dependent and ATP-dependent transport systems analogous to the bacterial "membrane-bound" and "binding protein" systems (25). Despite some uncertainties, we consider our results to agree with the H^+ co-transport hypothesis, and in particular, our results support the less well substantiated part of the hypothesis that requires active, electrogenic H^+ extrusion in the continued presence of the solute.

Metabolic inhibitors are expected to reduce the proton motive force across the cell membranes (9, 19, 21), and the reduction in the amino acid-induced depolarizations (Fig. 4) is consistent with that expectation. The virtual elimination of the spontaneous repolarization by inhibitors supports the view that the repolarizations were a result of an active H^+ extrusion.

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