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Hydrogen Ion Cotransport by the Renal Brush Border Glutamate Transporter†

Pamlea J. Nelson, Gary E. Dean, Peter S. Aronson, and Gary Rudnick*

ABSTRACT: Sodium ion dependent glutamate transport into rabbit renal brush border membrane vesicles is stimulated by low external pH and inhibited by low internal pH. Imposition of a pH difference (ΔpH) (interior alkaline) across the vesicle membrane drives glutamate accumulation in the absence of other driving forces. This process requires Na^+ but is not due to generation of an Na^+ gradient in response to ΔpH. Internal K^+ stimulates both the rate and extent of glutamate accumulation, although K^+ is not absolutely required for transport. Internal H^+ inhibits the rate of glutamate accumulation by decreasing this K^+ stimulation. Conversely, external K^+ inhibits glutamate influx, and this inhibition can be overcome by lowering the external pH. These results indicate that H^+ is cotransported with glutamate and suggest the possibility that, in the absence of internal K^+, H^+ can also fulfill the requirement for a countertransported ion.

The dicarboxylic amino acid glutamate is the substrate for specific transport systems in kidney and brain. In brain, the action of glutamate as a neurotransmitter is believed to be terminated by rapid and efficient reuptake into presynaptic nerve terminals (Curtis et al., 1960). Brain slices and synaptosomes accumulate glutamate by a high-affinity process (Navon & Lathja, 1969; Balcar & Johnson, 1972; Logan & Snyder, 1972; Wheeler & Hollingsworth, 1978) that has also been studied in cultured cerebellar cells (Stallcup et al., 1979) and in isolated synaptic plasma membrane vesicles (Kanner & Sharon, 1978; Kanner & Marva, 1982). In the kidney, glutamate (like other amino acids) is freely filtered and extensively reabsorbed in the proximal convoluted tubule (Weber, 1962). Glutamate is rapidly transported into intact proximal tubule epithelial cells (Siebernagl et al., 1975; Samarzija & Fromter, 1975) and into renal brush border membrane vesicles (Weiss et al., 1978; Schneider et al., 1980; Burckhardt et al., 1980).

In many ways, glutamate transport is similar in brain and kidney. In both cases, glutamate influx involves Na^+ cotransport and K^+ countertransport (Schneider & Sacktor, 1980; Kanner & Sharon, 1978), the K_M for transport is in the micromolar range, and aspartate is a potent competitive inhibitor (Schneider et al., 1980; Stallcup et al., 1979). These similarities suggest that the same transporter catalyzes glutamate flux in both tissues and that the renal system might therefore provide a good model system for studying neuronal glutamate reuptake.

We first demonstrated countertransport with K^+ for the serotonin transporter of platelet plasma membrane (Rudnick, 1977; Rudnick & Nelson, 1978). This similarity between glutamate and serotonin transport suggested that other aspects of the transport mechanism might also be similar, especially since both compounds are neurotransmitters. In particular, we were interested in the observation that internal H^+ substitutes for K^+ in the serotonin system (Keyes & Rudnick, 1982). Schneider & Sacktor (1980) had reported that low pH also antagonized the stimulation of glutamate transport by internal K^+ and that glutamate transport was electroneutral both in the presence and absence of internal K^+. In the present paper, we have examined in greater detail the effects of internal and external pH on glutamate transport by renal microvillus membrane vesicles. Our results are consistent with a model in which Na^+, glutamate, and H^+ are cotransported in exchange for K^+ or H^+.

Experimental Procedures

Preparation of Membrane Vesicles. Microvillus membrane vesicles were isolated from rabbit renal cortex by the magnesium aggregation method described previously (Aronson, 1978). The homogenization and isolation medium consisted of 300 mM mannitol, 10 mM Tris,[1] and 16 mM Hepes, pH 7.5. Membranes isolated by this method are purified 14-fold in the luminal membrane marker enzyme, γ-glutamyltranspeptidase, and are depurified in the basolateral marker, Na^+,K^+-ATPase (Aronson, 1978).

Equilibration of Membrane Vesicles. Suspensions of vesicles (6–10 mg of membrane protein/mL by the Lowry assay with BSA as a standard) were diluted at least 10-fold into media of indicated composition (see figure legends) and incubated for 15 min at 37 °C. The suspension was then collected by centrifugation at 48000*g* for 20 min at 4 °C, resuspended in fresh medium of the same composition at 3 times the original protein concentration, and stored at 4 °C until

† From the Departments of Pharmacology (P.J.N., G.E.D., and G.R.), Medicine (P.S.A.), and Physiology (P.S.A.), Yale University School of Medicine, New Haven, Connecticut 06510. *Received June 3, 1983.* P.S.A. and G.R. are established investigators of the American Heart Association. This work was supported by U.S. Public Health Service Grants HL-21217 (to G.R.) and AM-17433 (to P.S.A.).

[1] Abbreviations: ΔpH, the pH difference across the membrane; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; BSA, bovine serum albumin; FCCP, carbonyl cyanide *p*-(trifluoromethyl)phenylhydrazone; Mops, 4-morpholinepropanesulfonic acid; ADA, *N*-2-acetamidoiminodiacetic acid.

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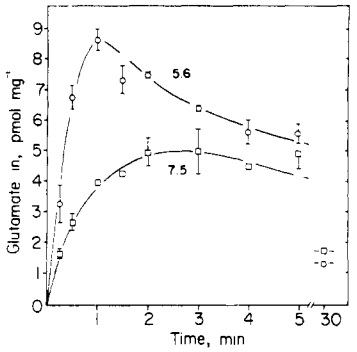

FIGURE 1: Stimulation of glutamate accumulation by external acidity. Vesicles were equilibrated in 92 mM KCl containing 4.2 mM potassium ADA buffer, pH 7.5, and 1 mM $MgSO_4$ and diluted 10-fold into either 92 mM NaCl containing 4.2 mM sodium ADA buffer, pH 7.5, and 1 mM $MgSO_4$ (squares) or 92 mM NaCl containing 7.3 mM sodium ADA buffer, pH 5.6, and 1 mM $MgSO_4$ (circles). The dilution solution also contained 0.15 μM L-[^{3}H]glutamate. Transport was stopped at the indicated times by dilution, filtration, and washing as described under Experimental Procedures.

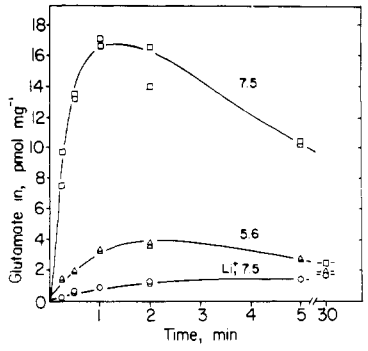

FIGURE 2: Stimulation of glutamate accumulation by K^+ and internal alkalinity. Vesicles were equilibrated in either 92 mM KCl containing 7.3 mM potassium ADA buffer, pH 5.6, and 1 mM $MgSO_4$ (triangles) or 92 mM LiCl containing 4.2 mM lithium ADA buffer, pH 7.5, and 1 mM $MgSO_4$ (circles). Some of the vesicles equilibrated in KCl at pH 5.6 were subsequently equilibrated at pH 7.5 (squares) by 10-fold dilution into 92 mM KCl containing 4.2 mM potassium ADA buffer, pH 7.5, and 1 mM $MgSO_4$ followed by a rapid freeze–thaw cycle and 3-fold dilution with the same buffer. These vesicles were incubated for 5 min at 37 °C and then collected by centrifugation and resuspended in their original volume of KCl buffer at pH 7.5. After equilibration, the vesicle suspensions were diluted 10-fold into 92 mM NaCl containing 7.3 mM sodium ADA buffer, pH 5.6, 1 mM $MgSO_4$, and 0.15 μM L-[^{3}H]glutamate, and transport was measured as described under Experimental Procedures.

used. In preliminary experiments, we found that the transport effects of equilibrating vesicles in various experimental media did not become greater with incubation times longer than 15 min at 37 °C; and thus, it was assumed that ionic equilibration had occurred during this interval.

Transport Assays. All transport assays were performed at 25 °C, and all solutions except for membrane suspensions were filtered through Gelman GN-6 filters before use to remove any contaminating microorganisms. A sample of 5 μL of preequilibrated membrane vesicles was diluted into 45 μL of the indicated solution (see figure legends) containing 0.15 μM L-[^{3}H]glutamate (Amersham, 10000 cpm/pmol). After the indicated time, the reaction mixture was rapidly diluted with 2 mL of ice-cold 167 mM NaCl, filtered through a 25-mm Gelman GN-6 cellulose nitrate filter, and washed with a second 2-mL portion of 167 mM NaCl. Dilution, filtration, and washing took less than 15 s. The filters were then dried and counted in toluene-based scintillation fluid at approximately 15% efficiency. The experimental glutamate uptake values were corrected for the nonspecific retention of glutamate by the cellulose nitrate filters. This was estimated from assays in which the first 2-mL portion of ice-cold diluting solution was added to the vesicles prior to the 45 μL of solution containing L-[^{3}H]glutamate. The final results are expressed as picomoles of L-glutamate uptake per milligram of membrane protein. For the experiments shown in Figures 4–7, controls in which Li^+ replaced Na^+ were subtracted from all experimental points.

Results

Time Course of Glutamate Transport. The data in Figure 1 demonstrate that external acidity stimulates glutamate transport by rabbit kidney brush border membrane vesicles. As previously demonstrated by Schneider et al. (1980), vesicles containing K^+ rapidly accumulate glutamate when diluted into Na^+ medium. This accumulation is driven by gradients of Na^+ and K^+ imposed by dilution and lasts only for the time that the gradients remain. When the vesicles are diluted into medium with a pH (5.6) lower than that of the vesicle lumen (7.5), glutamate enters rapidly and reaches a peak internal concentration within 1 min. In contrast, vesicles diluted into medium of the same pH (7.5) take up glutamate more slowly and reach a peak only after 2 min. The stimulatory effect of external acidity observed here cannot be attributed solely to inhibition of the rate of Na^+ gradient dissipation via Na^+–H^+ exchange (Kinsella & Aronson, 1980) since the initial rate of glutamate transport is also stimulated (see Figure 5).

In spite of the stimulation by low external pH, internal acidity inhibits glutamate influx. Figure 2 shows the results of an experiment in which vesicles were preequilibrated in KCl solutions at either pH 5.6 or 7.5 and then diluted into NaCl medium at pH 5.6. At the more alkaline internal pH, glutamate accumulation again reaches a maximal value within 1 min, in response to the imposed ion gradients, followed by loss of internal glutamate as those gradients decay. Lowering the internal pH to 5.6 inhibits this process over 80%. This inhibition cannot result from irreversible inactivation of the transport system since both vesicle suspensions had been equilibrated at pH 5.6. Part of this suspension was then washed and reequilibrated at pH 7.5 to obtain the results shown in the upper curve. Figure 2 also demonstrates the stimulation by internal K^+. When Li^+ replaces internal K^+, very little glutamate accumulates in the vesicles (bottom curve), even though the internal pH is high. Other experiments (data not shown) indicate that Li^+ does not replace Na^+ and, therefore, does not eliminate external Na^+ as a driving force. Again, as will be shown below (Figure 7), internal acidity inhibits the initial rate of glutamate influx, an effect that cannot be simply attributed to slowing the Na^+ gradient dissipation.

Transport Is Driven by ΔpH. Since low external pH stimulated transport and low internal pH inhibited, we tested the ability of an imposed ΔpH (interior alkaline) to drive glutamate transport in the absence of other driving forces. The data shown in Figure 3 demonstrate that ΔpH stimulates glutamate accumulation and that this stimulation requires Na^+. Vesicles were equilibrated with Na^+, K^+, and glutamate at pH 7.5 for 1 h at 25 °C, by which time all solutes were at equilibrium across the membrane. At this time, addition of enough Mes free acid (final concentration, 10.5 mM) to lower the external pH to 5.6 resulted in the transient accumulation of glutamate over the equilibrium level (open circles). This overshoot requires Na^+ since Li^+ fails to support influx in response to ΔpH (filled circles). Thus ΔpH does not drive glutamate transport simply by nonionic diffusion of the free acid or by Na^+-in-

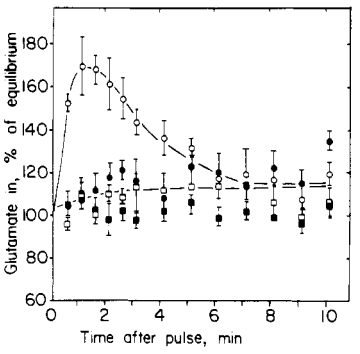

FIGURE 3: Glutamate accumulation is driven by ΔpH. Vesicles were equilibrated either with 92 mM NaCl containing 4.2 mM sodium ADA buffer, pH 7.5, 1 mM KCl, 132 mM mannitol, and 1 mM $MgSO_4$ (open symbols) or the same solution with lithium replacing sodium (filled symbols). The presence of potassium was required to demonstrate the effect of a pH gradient under these conditions. After equilibration, the suspensions were diluted with equilibration buffer to a final protein concentration of 2 mg/mL, and 0.15 μM L-[^{3}H]-glutamate was added. After incubation for 1 h at 25 °C, a sufficient volume (1% of the total reaction mixture) of 1 M Mes free acid (circles) was added to lower the extravesicular pH to 5.6. In the controls, the same volume of 1 M Mes adjusted to pH 7.5 with NaOH was added (squares). Samples containing 100 μg of membrane protein were removed at the indicated times, diluted, filtered, and washed as described under Experimental Procedures. Values are expressed as percent of equilibrium glutamate content (before Mes addition) and are averages, plus or minus standard error, of five separate experiments with three separate membrane preparations. Average equilibrium glutamate content was 0.437 pmol/mg in Na^+ and 0.451 pmol/mg in Li^+ medium.

dependent organic anion–hydroxide exchange, as has been described in dog renal microvillus membranes (Blomstedt & Aronson, 1980).

One possible mechanism by which ΔpH could stimulate glutamate influx is through the action of the Na^+–H^+ exchange activity of these membranes (Kinsella & Aronson, 1980). If ΔpH (interior alkaline) resulted in generation of an Na^+ gradient (out > in), transport would have been energized only indirectly by ΔpH. To test this possibility, we added an equivalent amount of Mes buffered to pH 7.5 with 10.5 mM NaOH instead of the free acid. As shown in Figure 3 (open squares), imposition of an Na^+ gradient at least as large as that expected from Na^+–H^+ exchange failed to drive glutamate influx in the absence of ΔpH. The same result was observed when Li^+ replaced Na^+ (filled squares). These results strongly suggest direct coupling of glutamate accumulation to ΔpH, as would be expected for H^+–glutamate cotransport.

Effect of K^+ and H^+ on Glutamate Influx. Just as internal K^+ stimulates glutamate influx (Figure 2) (Schneider et al., 1980), external K^+ inhibits. The data in Figure 4 demonstrate the effect of external K^+ on the rate of glutamate accumulation at two external pH values. At pH 7.5, external K^+ strongly inhibits glutamate influx. The rate is 50% inhibited at approximately 10 mequiv of K^+/L and approximately 90% inhibited at 90 mequiv/L. At an external pH of 5.6, however, influx is faster, and K^+ is a much weaker inhibitor. The rate of glutamate transport is inhibited by 50% only at approximately 90 mequiv of K^+/L. Thus, higher concentrations of the cotransported ion, H^+, are required to overcome inhibition by the countertransported ion, K^+.

The experiment shown in Figure 5 demonstrates that K^+ shifts the pH rate profile for glutamate transport to more acid pH. Both in the presence and in the absence of K^+, decreasing the external pH increases glutamate influx. In neither case does the rate saturate at low pH, which suggests a low pK_a for the site to which H^+ binds prior to cotransport.

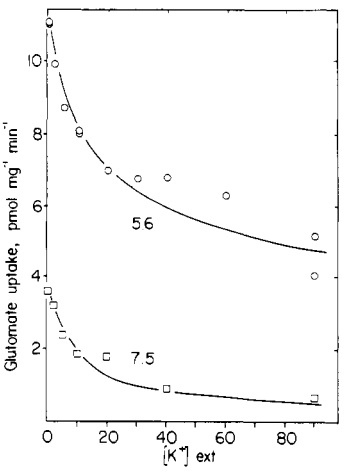

FIGURE 4: Inhibition of glutamate influx by external K^+. Vesicles were equilibrated in 67 mM KCl containing 90 mM LiCl, 1 mM $MgSO_4$, 4 mM mannitol, and 4.2 mM potassium ADA buffer, pH 7.5. They were assayed in media of the indicated K^+ concentration with LiCl added to maintain constant alkali cation concentration either at pH 5.6 (circles) or at 7.5 (squares). Dilution media consisted of 67 mM NaCl, 90 mM KCl (or LiCl), 1 mM $MgSO_4$, 4 mM mannitol, 0.15 μM L-[^{3}H]glutamate, and either 4.2 mM sodium ADA buffer, pH 7.5, or 7.3 mM sodium ADA buffer pH 5.6. Transport was measured at 15 s, which represented the initial rate.

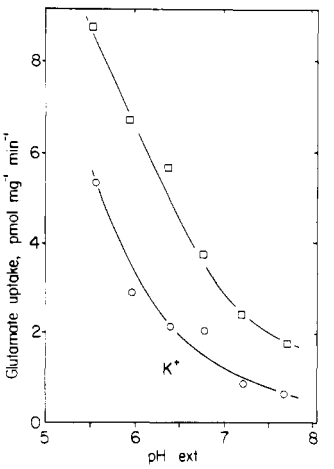

FIGURE 5: External pH–rate profile for glutamate influx. Vesicles equilibrated in the same solution as described in the legend to Figure 4 were diluted into 67 mM NaCl containing either 90 mM LiCl (squares) or 40 mM KCl plus 50 mM LiCl (circles), 1 mM $MgSO_4$, 5 mM Mes, 5 mM Mops adjusted to the indicated pH with LiOH, enough mannitol to adjust the final osmolarity to 330 mOsm, and 0.15 μM L-[^{3}H]glutamate. Initial rates of transport were measured at 15 s.

Achievement of any given transport rate requires, however, a lower pH in the presence of K^+ than in the absence, as if H^+ and K^+ were in competition with one another.

The concentration dependence for stimulation by internal K^+ is shown by the data in Figure 6. Again the effects of H^+ and K^+ are mutually antagonistic. Lowering the internal pH from 7.5 to 6.0 markedly attenuates the stimulation by K^+ at essentially all K^+ concentrations, just as low external pH attenuated the inhibitory effect of external K^+ (Figure 4). It is noteworthy that transport is still observed in the total absence of internal K^+. All of the rates shown are corrected for Na^+-independent influx. Thus, residual glutamate influx in the absence of K^+ still absolutely requires external Na^+.

The internal pH rate profile for glutamate transport also demonstrates a marked dependence on internal K^+. The data in Figure 7 show the dramatic increase in glutamate influx into K^+-containing vesicles as the internal pH is raised from

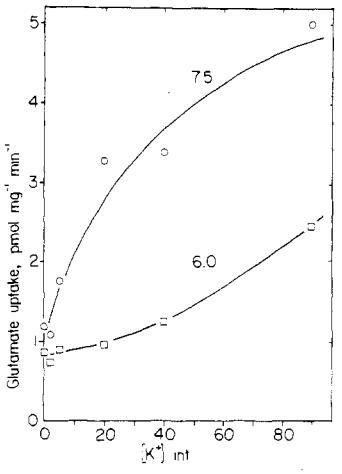

FIGURE 6: Stimulation of glutamate influx by internal K^+. Vesicles were equilibrated in 67 mM LiCl plus the indicated concentration of KCl with LiCl added to maintain constant alkali cation concentration at either pH 7.5 (circles) or 6.0 (squares). The solutions also contained 1 mM $MgSO_4$, 5 mM Mes, 5 mM Mops, enough LiOH to adjust pH to the indicated value, and enough mannitol to maintain constant osmolarity (330 mOsm). The vesicle suspension was diluted into 67 mM NaCl containing 90 mM LiCl, 1 mM $MgSO_4$, 7.75 mM mannitol, 5 mM Mes, 5 mM Mops, pH 6.0, and 0.15 μM L-[^{3}H]-glutamate. Initial rates of transport were measured at 15 s.

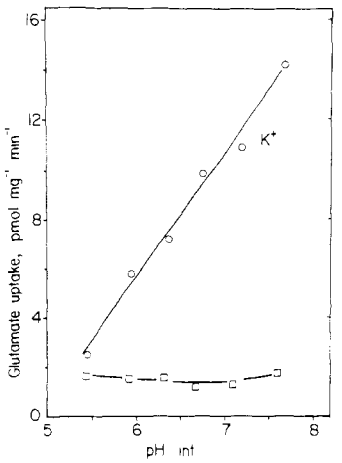

FIGURE 7: Internal pH–rate profile for glutamate influx. Vesicles were equilibrated in either 157 mM LiCl (squares) or 90 mM KCl plus 67 mM LiCl (circles) at the indicated pH. The solutions also contained 5 mM Mes, 5 mM Mops, adjusted to the indicated pH with LiOH, 1 mM $MgSO_4$, and a sufficient amount of mannitol to maintain constant osmolarity (330 mOsm). The vesicle suspension was diluted into 67 mM NaCl containing 90 mM LiCl, 1 mM $MgSO_4$, 7.75 mM mannitol, 5 M Mes, 5 mM Mops, pH 6.0, and 0.15 μM L-[^{3}H]-glutamate. Initial rates of transport were measured at 15 s.

5.6 to 7.5. Internal H^+ again inhibits stimulation by internal K^+. In contrast, there is little or no effect of internal pH in the absence of K^+. Thus, internal H^+ inhibits only the K^+-stimulated rate, not the transport observed in the absence of K^+.

Discussion

The results presented here provide direct evidence for coupling between glutamate and H^+ fluxes by the Na^+-dependent glutamate transporter of renal brush border membrane vesicles. The strongest evidence supporting glutamate–H^+ cotransport is the ability of a pH difference (ΔpH) across the vesicle membrane to drive net glutamate accumulation in the absence of other energy sources (Figure 3). Furthermore, H^+ has predictable effects on the rate of glutamate accumulation, stimulating externally (Figures 1, 4, and 5) and inhibiting

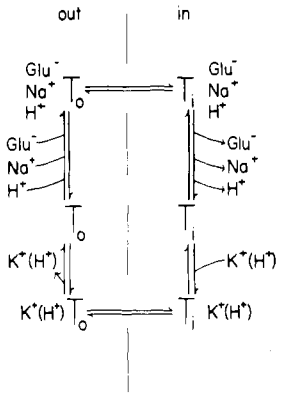

FIGURE 8: Possible mechanism for glutamate transport. T_i and T_o represent two forms of the transporter with substrate binding sites exposed to the internal and external surface of the membrane, respectively. Interconversion of the two forms occurs either with Na^+, H^+, and glutamate bound (upper pathway) or with a cation (usually K^+ but possibly also H^+) bound (lower pathway).

internally (Figures 2, 6, and 7). These results favor a transport mechanism similar to the simplified scheme shown in Figure 8.

In this model, glutamate, Na^+, and H^+ bind to the transporter on the external surface of the membrane and are subsequently translocated to the interior surface, where they dissociate. To catalyze more glutamate accumulation, the transporter must return to a form capable of binding external substrates. This transformation is usually accomplished by binding intracellular K^+, translocating it to the external membrane surface, and releasing K^+ to the external medium. The scheme thus accounts for glutamate–H^+ cotransport and also the observation by Schneider & Sacktor (1980) that internal K^+ stimulates glutamate accumulation. In that report, Schneider and Sacktor noted that simultaneously raising both internal and external H^+ concentration prevented much of the K^+ stimulation. They suggested that the inhibitory effect was mediated by internal H^+ and proposed glutamate–H^+ cotransport as a possible explanation.

Our results confirm the proposal by Schneider & Sacktor (1980) and provide a basis for understanding the interaction between H^+ and K^+. Looking at the transport process as an enzymatic reaction, we see that internal K^+ and external H^+ are substrates, while external K^+ and internal H^+ are products. These products inhibit the forward reaction. Figures 4 and 5 demonstrate inhibition by external K^+ while Figures 2, 6, and 7 show that internal H^+ inhibits. Product inhibition may result from reversal of the overall reaction (stimulation of glutamate efflux) or from a simple favoring of the transporter–product complex which prevents formation of the proper transporter–substrate complex.

This second possibility can explain the apparent competition between H^+ and K^+. The scheme drawn in Figure 8 shows H^+ and K^+ competing for free transporter on both the internal and external surfaces of the membrane. It is consistent with the observations that product inhibition by internal H^+ (Figures 6 and 7) is overcome by higher concentration of internal substrate (K^+) and product inhibition by external K^+ (Figures 4 and 5) is overcome by higher concentrations of external substrate (H^+). Although our results fit the model shown in Figure 8, they do not exclude some other possibilities, such as K^+ countertransport simultaneous with glutamate translocation or the existence of transporter species with both H^+ and K^+ bound on the same face of the membrane.

The results shown in Figures 6 and 7 clearly demonstrate Na^+-dependent glutamate influx in the absence of added in-

ternal K^+. Moreover, internal H^+ fails to inhibit transport into K^+-free vesicles (Figure 7). This observation raises the possibility that H^+ can replace K^+ as a countertransported cation and that subsequent return of the transporter to T_o is much slower with H^+ than with K^+. In this case, we would predict that the pK_a for the internal cation site is high enough that it is almost completely saturated with H^+ at the alkaline end of the pH range studied (Figure 7). Internal K^+ would accelerate glutamate influx by binding to the small unprotonated fraction of the transporter followed by rapid translocation. Thus, K^+ stimulation is expected to continue to increase with internal pH, as demonstrated by the results shown in Figure 7. The predicted high pK_a of the internal cation site contrasts with the apparent low pK_a of the external cation site (Figure 5). Possible explanations for this apparent asymmetry include an intrinsic asymmetry in the transporter structure, an asymmetry imposed by the Na^+ gradient, or the existence of two separate cation sites for co- and countertransport. Indeed, it is possible that the low-pK external H^+ site is the γ-COOH of glutamate ($pK_a = 4.25$) so that glutamic acid is actually the transported moiety. However, such a model could not explain the apparent competition between external K^+ and H^+. This competitive interaction suggests that glutamate and H^+ are cotransported while bound to separate sites and that the anionic form, rather than free glutamic acid, is the true substrate for the transporter.

We have previously shown that the platelet plasma membrane serotonin transporter, which is also stimulated by internal K^+ (Rudnick & Nelson, 1978; Nelson & Rudnick, 1979), countertransports H^+ in the absence of internal K^+ (Keyes & Rudnick, 1982). In the platelet system, serotonin accumulation is stimulated by a ΔpH (interior acid), but only in the absence of K^+. This contrasts with the stimulation of glutamate transport by a ΔpH of the opposite polarity (interior alkaline). In spite of this difference, these two distinct transporters are strikingly similar since, in both cases, H^+ and K^+ compete with each other.

One question that remains unresolved is whether charge is translocated with glutamate. Schneider et al. (1980) and Schneider & Sacktor (1980) generated electrical potentials across the brush border membrane by using K^+ gradients and valinomycin, H^+ gradients and FCCP, or gradients of permeant anions. They observed little effect of membrane potential on transport and concluded that the process was electroneutral both in the presence and absence of internal K^+. Burckhardt et al. (1980) used exactly the same techniques and reached the opposite conclusion, namely, that glutamate was transported with net positive charge and that the transport process was electrogenic only when intravesicular K^+ was present. Kanner & Sharon (1978) also observed stimulation of a K^+ diffusion potential in the glutamate transporter from rat brain. One possible problem associated with these studies is that both K^+ and H^+, the two ions used to generate a membrane potential, are also substrates for the transport system. If our suggestion that H^+ replaces K^+ as a countertransported ion proves correct, however, it will render unlikely any change in electrogenicity when internal K^+ is removed.

The simplest stoichiometry consistent with the data presented here is the one shown in Figure 8, namely, that Na^+, H^+, and glutamate are cotransported and K^+ is countertransported in a 1:1:1:1 stoichiometry. In the absence of internal K^+, we would predict that H^+ would recycle and the transporter would catalyze net Na^+-glutamate cotransport (1:1). However, many workers have suggested that more than one Na^+ is cotransported with glutamate from measurements of electrogenicity (Kanner & Sharon, 1978; Burckhardt et al., 1980), kinetics (Wheeler & Hollingsworth, 1978), and Na^+ flux (Stallcup et al., 1979). These observations are not inconsistent with any of the results reported here, but future proposals concerning the transport stoichiometry must take H^+-glutamate cotransport into account.

Acknowledgments

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Registry No. K, 7440-09-7; Na, 7440-23-5; hydrogen ion, 12408-02-5; L-glutamic acid, 56-86-0.

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