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The Kinetic Mechanism of the Glutamate–Aspartate Carrier in Rat Intestinal Brush-Border Membrane Vesicles: The Role of Potassium

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The sodium dependent transport system for L-glutamate and L-aspartate localized in the apical part of rat enterocytes has previously been kinetically characterized (Prezioso, G., and Scalera, V. (1996). *Biochim. Biophys. Acta* **1279**, 144–148). In this paper the mechanism by which the potassium cation specifically activates the L-glutamate–sodium cotransport process is investigated. Potassium has been found to act as an activator when it is present inside the membrane vesicles, while its presence outside is ineffective, and the effect is saturable. The kinetic parameters with respect to sodium and glutamate have been compared in the presence and in the absence of the activator. The results indicate that the ordered sodium–sodium glutamate mechanism is not altered by potassium, and that the activation is probably exerted on both the rate determining steps of the transport process. It is proposed that (1) a specific binding site for potassium is present on the inside hydrophilic part of the membrane carrier, (2) the binding of the effector accelerates the intramembrane rearrangement steps of both the disodium glutamate–carrier complex and the free carrier, (3) the affinity of the carrier is lowered with respect to sodium whereas it is increased for glutamate, and (4) K^+ antiport is not performed by this carrier.

KEY WORDS: Glutamate; transport; sodium ion cotransport; potassium; brush-border membrane vesicles; kinetics; rat intestine.

INTRODUCTION

The brush-border membrane of rat enterocytes contains a sodium dependent transport system specific for the acidic amino acids L-glutamate and L-aspartate (Corcelli *et al.*, 1982). Its kinetic characterization by the use of isolated brush-border membrane vesicles (BBMV) led to the conclusion that it is a low affinity transport system, operating a cotransport of two sodium ions and a glutamate (or aspartate) molecule, by a sequential ordered mechanism (Prezioso and Scalera, 1996). It is also known that the K^+ cation, when present inside the vesicles, specifically activates the sodium dependent glutamate uptake in rat intestinal BBMV (Corcelli and Storelli, 1983), as well as in proximal tubular BBMV (Heinz *et al.*, 1988). The renal glutamate transport system has been shown to counter-exchange potassium with sodium and glutamate, while, in the intestinal transport system, only the potassium activating effect has been reported.

This paper deals with the mechanism by which the potassium cation exerts its activating effect on the glutamate transport in rat enterocyte BBMV, by means of the kinetic approach already used to characterize this transport system (Prezioso and Scalera, 1996).

MATERIALS AND METHODS

Chemicals

L-[^{3}H]glutamate was obtained from Amersham Italy; all chemicals used were of analytical grade purity.

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Preparation of Membrane Vesicles

Intestinal brush-border membrane vesicles (BBMV) were isolated as described previously (Prezioso and Scalera, 1996). Briefly, Sprague Dawley rats were killed by decapitation and the intestines were removed immediately. After washing twice with ice-cold 0.9% NaCl solution containing 0.02% NaN_3, they were everted and scraped. The scraped mucosa was homogenized, and the BBMV were prepared by Mg^{++}/EGTA precipitation and differential centrifugation according to Hauser *et al.* (1980). All steps of the preparation of the BBMV were performed at 4°C and all suspension media were supplemented with 1 mM PMSF (phenyl-methyl-sulfonyl-fluoride). Membrane vesicles obtained were suspended in 100 mM Mannitol, HEPES/Tris 20 mM (pH 7.5). The specific activity of alkaline phosphatase (EC 3.1.3.1) was typically enriched 20-fold in the brush-border fraction as compared to the initial homogenate (Scalera *et al.*, 1980).

Protein Determination

Protein content was determined by the Bradford method (Bradford, 1976) using the Bio Rad kit (Bio Rad, Richmond, CA, U.S.A.) and γ-globulin as a standard. The protein concentration of the vesicle preparation was measured before the uptake was started, and adjusted so that 90 μg of vesicle membrane protein per experimental point was used.

Uptake Assays

Uptake assays were carried out using the rapid filtration technique as described elsewhere (Hopfer *et al.*, 1973; Scalera *et al.*, 1987). Briefly, 6 μL of membrane vesicle suspension (equivalent to 90 μg of membrane protein) was added to 24 μL of incubation medium. At the time indicated, the incubation was stopped by diluting the sample with 1 mL of ice-cold isotonic stop solution containing 150 or 250 mM NaCl, 20 mM HEPES/Tris pH 7.5. The membrane vesicles were immediately collected on a wetted millipore-filter (0.45 μm pore size) and washed once with 5 mL of the above NaCl solution to remove the unspecific radioactivity bound. L-[^{3}H]glutamate trapped on the filters was measured in a liquid scintillation counter (Beckman). Membrane free incubation media were used as blanks. For each L-[^{3}H]glutamate concentration, the counts of the samples were at least 4 times higher than those of the blanks. Each experiment was performed at least 4 times, and only the results of a typical experiment are shown. All experimental data reported are the average of triplicate assays. Experimental scatter of triplicates was always less than 10% of the mean values. Uptake rates are generally expressed as pmol/mg protein/min. In all experiments the incubation time was 7 s, since in preliminarily performed control assays the L-glutamate uptake (measured both in the presence and in the absence of NaCl) was linear for at least 10 s of incubation for all the L-glutamate concentrations used. The kinetics of the uptake were analyzed using linear and nonlinear regression procedures with the aid of a personal computer. Statistical analysis was determined using the Student's *t* test for unpaired data ($P < 0.05$ was considered statistically significant).

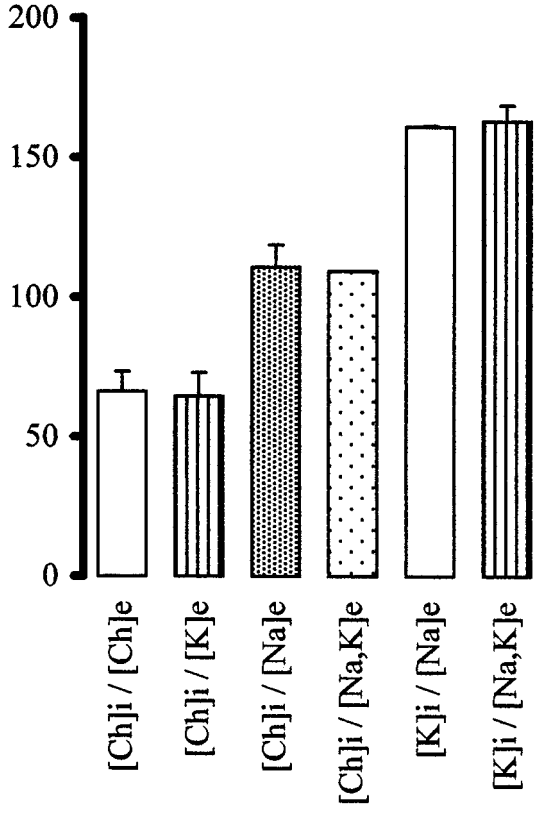

Fig. 1. Effect of potassium on L-glutamate transport. Membrane vesicles were suspended in two alternative media, containing choline ($[Ch]_i$) or potassium ($[K]_i$) with the following composition; 200 mM ChCl, 100 mM Mannitol, 20 mM Hepes/Tris (pH 7.5), and 100 mM KCl, 100 mM ChCl, 100 mM Mannitol, 20 mM Hepes/Tris (pH 7.5), respectively (final concentrations). The uptake was started by adding vesicles to four different incubation media all containing 100 mM Mannitol, 20 mM Hepes/Tris (pH 7.5), 0.5 mM L-[^{3}H]glutamate, and one of following chloride salts as follows: 200 mM ChCl ($[Ch]_e$), 100 mM NaCl, 100 mM ChCl ($[Na]_e$), 100 mM NaCl, 100 mM KCl ($[Na, K]_e$), 100 mM KCl, 100 mM ChCl ($[K]_e$). Values represent means ± ES for triplicate samples.

RESULTS

A first analysis of the effect of potassium on the L-glutamate transport across rat enterocyte BBMV shows that the cation activates the transport process only when present on the internal side of the vesicles. As shown in Fig. 1, internally added potassium chloride at 100 mM concentration enhances the sodium dependent transport rate, while external potassium has no effect. In addition, when K^+ is present on both sides of the membrane, the glutamate transport rate is the same as that measured in the presence of the potassium gradient (in > out).

It is clear that the potassium activating effect does not require the presence of a gradient across the membrane, suggesting that K^+ is not transported by the carrier.

Figure 2 shows the dependence of the L-glutamate transport rate on the internal KCl concentration in the presence of 0.2 mM L-glutamate, at two different sodium concentrations. In both experimental conditions potassium stimulates the transport of glutamate showing hyperbolic saturation characteristics. It can be observed that the V_M is positively influenced by sodium concentration, while the $K_{0.5}$ values for potassium calculated in the presence of 40 and 100 mM NaCl are 19.1 ± 1.4 and 13.5 ± 0.3 mM, respectively. This result is indicative of a specific interaction of K^+ with the carrier molecule. Also taking into account the results of Fig. 1, it follows that a K^+ specific binding site must be localized on the inner face of the membrane. In this hypothesis, we have to consider that the free carrier molecule is in equilibrium with K^+ and the potassium bound carrier, and hence that, in the steady state of the Na^+ glutamate contransport process, each of the three possible forms of the carrier (free, sodium bound, and sodium glutamate bound) must be in equilibrium with the respective potassium bound form. Finally, in the presence of saturating K^+ concentrations, the potassium bound forms will greatly exceed the others. In the light of this information, we have reconsidered the kinetics of the substrate dependence of the transport that led to the definition of a mechanism for the process and some kinetic parameters (Prezioso and Scalera, 1996).

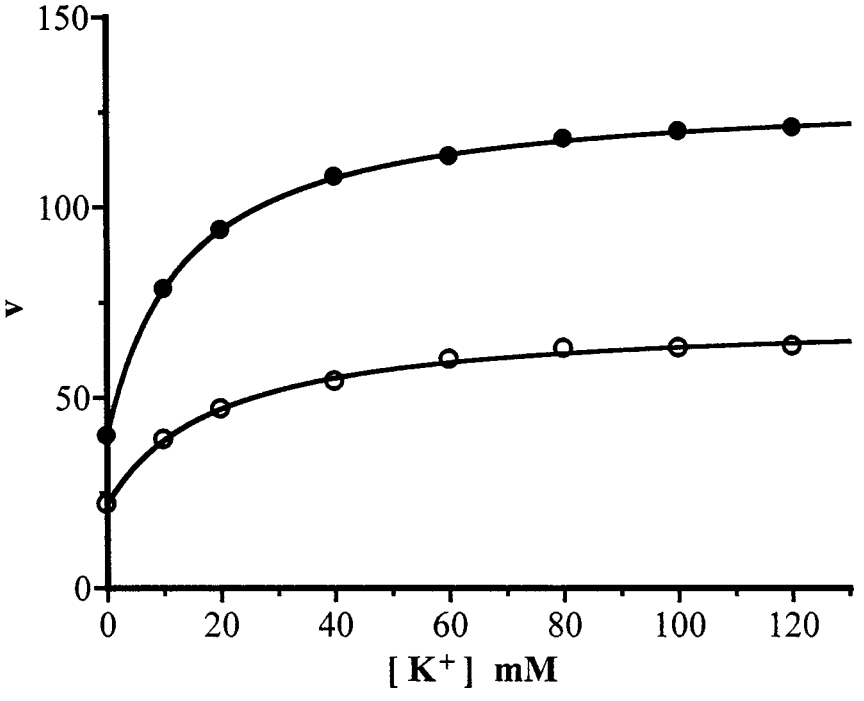

Fig. 2. Dependence of L-glutamate transport rate on internal potassium concentration. Membrane vesicles were suspended in incubation media containing 100 mM Mannitol and increasing KCl concentration in the range from 0 to 120 mM and ChCl enough to reach 200 mM total salt concentration buffered with 20 mM Hepes/Tris (pH 7.5). The uptake (7 s of incubation) was started by adding vesicles to media containing (final concentrations) 100 mM NaCl (•) or 40 mM NaCl, 60 mM ChCl (○) and 100 mM Mannitol, 20 mM Hepes/Tris (pH 7.5), 0.2 mM L-[^{3}H]glutamate. Transport rate is expressed as pmol/mg protein per min of L-[^{3}H]glutamate. All experimental data represent the average of triplicates.

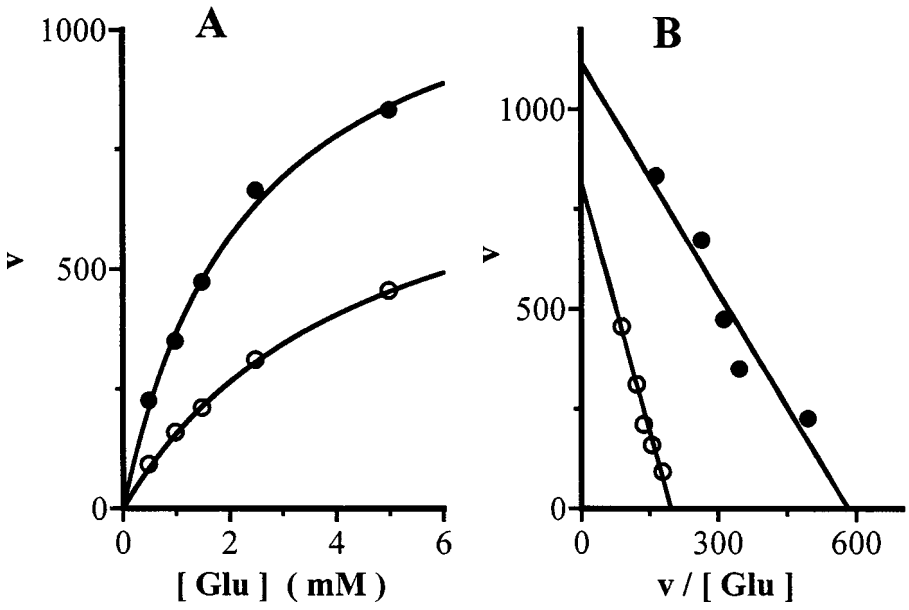

Fig. 3. Substrate concentration dependence of L-glutamate uptake. (A) Membrane vesicles were incubated in 200 mM ChCl (○) or 100 mM KCl, 100 mM ChCl (●) and 100 mM Mannitol, 20 mM Hepes/Tris (pH 7.5). The uptake (7 s) was started by adding vesicles to medium containing 100 mM Mannitol, NaCl 100 mM, 20 mM Hepes/Tris (pH 7.5) and L-[^{3}H]glutamate at the concentrations indicated. The reported values of L-glutamate transport rate are corrected for the sodium-independent component. (B) Eadie-Hofstee plot of the data. Transport rate V is expressed as pmol/mg protein per min.

The following experiments were performed in order to investigate how the substrate dependence of the glutamate transport activity is modified by the presence of potassium. Figures 3 and 4 show plots from two typical experiments of L-glutamate transport rate against to L-glutamate and sodium concentration, measured both in the absence and in the presence of 100 mM KCl (a concentration that can be considered saturating on the basis of Fig. 2). Each experiment is also reported as an Eadie-Hofstee plot, where the transport rate (v), measured at the substrate concentration S is reported against the v/S ratio. In such plots the intercept of the straight line on the vertical axis is the saturation velocity, while the slope is $-K_M$. As for the glutamate dependence (Fig. 3), it can be observed that the saturation velocity V_M (nmol/min/mg plot) was clearly enhanced by the presence of potassium (from 868 ± 29 to 1242 ± 68), while the experimental K_M for glutamate was lowered from 4.6 ± 0.3 to 2.4 ± 0.3 mM. At variable sodium concentration (Fig. 4), $K_{0.5}$ (the sodium semiactivation constant) was increased in the presence of potassium from 26 ± 0.45 to 32 ± 0.7 mM and V_M was again enhanced from 72 ± 1 to 112 ± 2.

It should to be noted that in the Eadie-Hofstee plots we report $[Na^+]^2$, instead of $[Na^+]$, on the basis of the results of the previous paper, where a stoichiometry of 2 Na^+ per carrier molecule was stated, with an experimental Hill number of 2. Actually, the straight lines in Fig. 4(B) demonstrate that the activator, whatever the mechanism of action, does not modify this aspect of the process.

A statistical treatment of a number of similar experiments provided a more precise evaluation of kinetics parameters (Table I). Because of the observed variability in the activity of different vesicle preparations, V_M values are given as percent of controls (absence of activator). The Student's t test was used to compare the parameters obtained in the absence and in the presence of potassium. The reported P values clearly show that the differences are statistically significant ($P < 0.01$).

A parallel evaluation of the Hill number from the same experiments used in Table I confirms the conclusion

Table I. Mean Values ± SE of Kinetic Parameters Obtained From Five Different Experiments the Same as Those in Figs. 3 and 4

	$V_{M,Glu}$ (% of control)	$K_{M,Glu}$ (mM)	$V_{M,Na}$ (% of control)	$K_{0.5,Na}$ (mM)
Control	100	4.1 ± 0.3	100	20.7 ± 1.9
$+K_i^+$	145 ± 11	$2.5 \pm 0.3^*$	157 ± 5	$30.2 \pm 1.6^*$

$^*P < 0.01$.

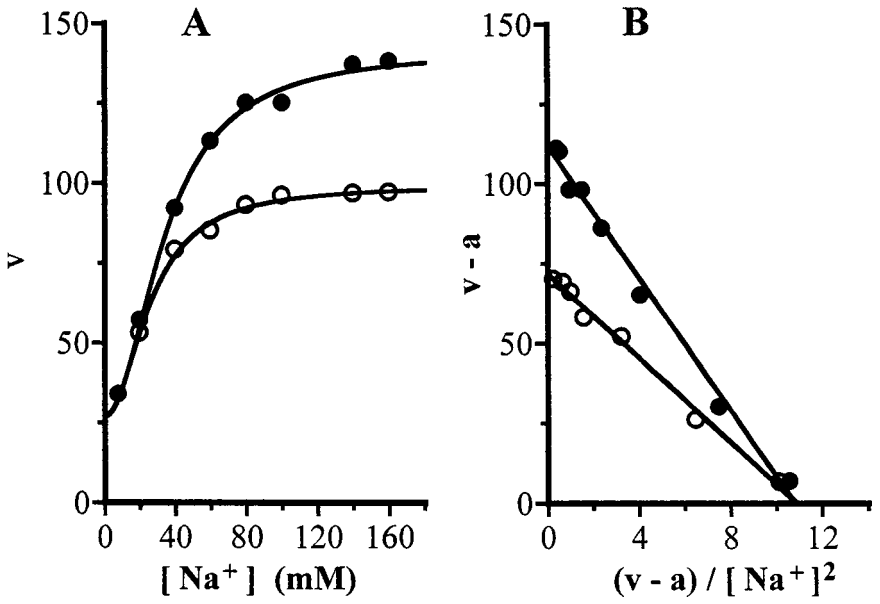

Fig. 4. Sodium concentration dependence of L-glutamate transport. (A) Membrane vesicles were incubated in 200 mM ChCl (○) or 100 mM KCl, 100 mM ChCl (●) and 100 mM Mannitol, 20 mM Hepes/Tris (pH 7.5). The uptake (7 s) was started by adding vesicles to medium containing 100 mM Mannitol, 1 mM L-[^{3}H]glutamate, 20 mM Hepes/Tris (pH 7.5) and NaCl at the concentration indicated. (B) Eadie-Hofstee type plot of the data corrected for the sodium-independent component. Transport rate V is expressed as pmol/mg protein per min.

of Fig. 4(B) that, both in the presence and in the absence of potassium, it is close to 2 (1.96 ± 0.08 in the presence of potassium; 1.91 ± 0.10 in its absence).

Of course, the K_M values so calculated are not the true ones, since they are functions of the concentration of the second substrate, that is kept constant in each plot, while only V_M values obtained from plots like those in Fig. 3 are actually concentration independent. In fact, the equation describing the initial transport rate as a function of glutamate and sodium concentrations, according to a sequential ordered mechanism (Prezioso and Scalera, 1996), in the absence of substrates inside the vesicles is

$$v = V'_M \cdot \frac{[Na]^2[Glu]}{K'_{Glu}K'_{Na} + K'_{Glu}[Na]^2 + [Na]^2[Glu]} \quad (1)$$

This equation can assume the forms

$$v_{Glu} = V'_M \cdot \frac{[Glu]}{K'_{Glu}\left(1 + \frac{K'_{Na}}{[Na]^2}\right) + [Glu]} \quad (2)$$

$$v_{Na} = \frac{V'_M}{1 + (K'_{Glu}/[Glu])} \cdot \frac{[Na]^2}{\frac{K'_{Na}}{1+([Glu]/K'_{Glu})} + [Na]^2} \quad (3)$$

that express the transport rate as a function of [Glu] and [Na^+] respectively and are used to fit the kinetic experimental data.

The concentration independent parameters, V'_M, K'_{Glu}, and K'_{Na} are defined in more detail in the Discussion section.

K'_{Glu} and K'_{Na} can be extrapolated from double substrate experiments. Figures 5 and 6 show the results of typical experiments of this kind, reported as Eadie-Hofstee plots in the absence and in the presence of potassium inside the vesicles, respectively. Figure 5 shows the expected pattern for the sequential ordered mechanism, with glutamate as second substrate. When [Glu] is the independent variable, the lines obtained at different sodium concentrations converge at one point on the v axis, i.e., corresponding to the concentration independent saturation velocity, V'_M (plot A). In plot B the lines at different [Glu] meet at a point on the left of the v axis, again at V'_M. A parallel experiment performed in the presence of internal K^+ is reported in Fig. 6. It is important to notice that the presence of the activator does not change the overall pattern. The sequential ordered mechanism is maintained, but of course the kinetic parameters are altered, as shown by their extrapolation. Second-order plots allow the evaluation of concentration independent K_M values for glutamate and

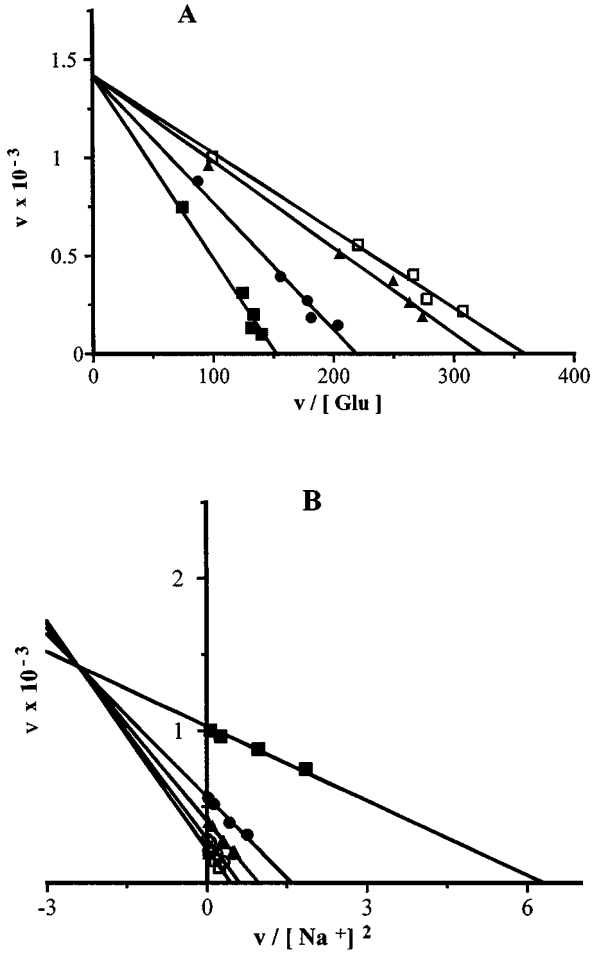

Fig. 5. Dependence of L-glutamate transport rate on bi-substrate concentration. Membrane vesicles were suspended in 200 mM ChCl, 100 mM Mannitol, 20 mM Hepes/Tris (pH 7.5). The uptake (7 s) was started by adding vesicles to five different media containing 0, 20, 30, 60, 120 mM NaCl, respectively, and appropriate ChCl concentrations, to achieve 200 mM total salt concentration, 20 mM Hepes/Tris (pH 7.5) and L-[^{3}H]glutamate. In each series L-glutamate concentration varied as follows: 0.7, 1, 1.5, 2.5, 10 mM (final concentrations). (A) Eadie-Hofstee plot of the L-glutamate transport rate versus V/[Glu] at 20 (■), 30 (●), 60 (▲), 120 (□) mM sodium. (B) Eadie-Hofstee plot of the L-glutamate transport rate versus $V/[Na^+]^2$ at 0.7 (□), 1 (○), 1.5 (▲), 2.5 (●), 10 (■) mM L-glutamate. Experimental values have been subtracted for the sodium-independent component. Transport rate V is expressed as pmol/mg protein per min.

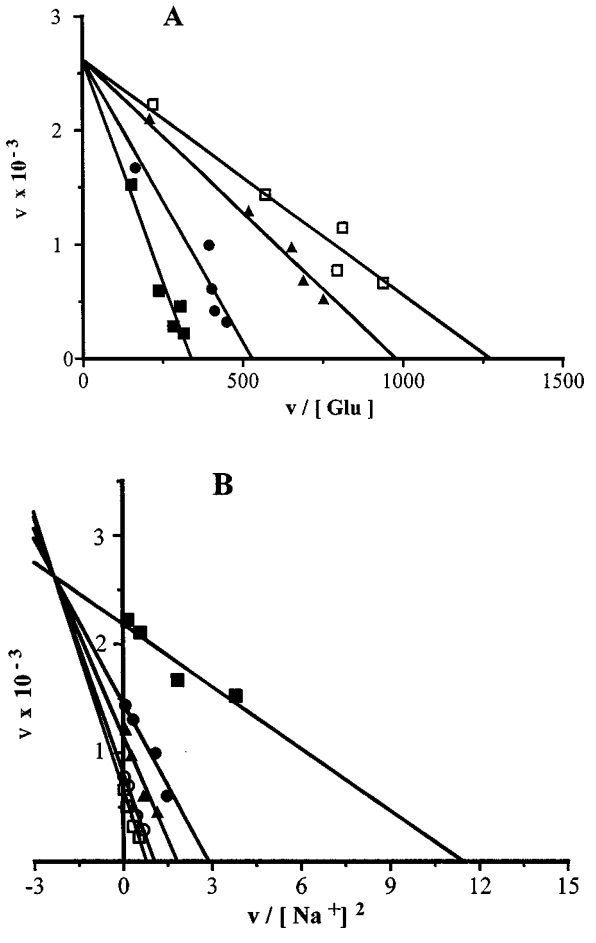

Fig. 6. Bi-substrate dependence of L-glutamate transport rate in the presence of potassium. Experimental conditions are the same as those for Fig. 5, except that membrane vesicles were preincubated in a medium containing 100 mM KCl, 100 mM ChCl, 100 mM Mannitol, 20 mM Hepes/Tris (pH 7.5).

sodium (Fig. 7), leading to a conclusion similar to that of Table I. In fact extrapolated K'_{Na} is significantly enhanced in the presence of internal potassium, while K'_{Glu} is lowered. Statistical evaluation of these parameters, based on a number of experiments, confirms these indications as shown in Table II ($P < 0.04$). These results allow us to conclude that potassium affect both K'_{Na} and K'_{Glu} in opposite directions.

DISCUSSION AND CONCLUSIONS

Equation (1) is obtained by adapting the Haldane procedure (Segel, 1975) to the transmembrane cotransport process, using the following scheme of kinetic mechanism based on the sequential ordered process as already stated

Table II. Mean Values ± SE of Extrapolated Dissociation Constants for Glutamate and Sodium, Obtained From Five Different Experiments the Same as Those in Figs. 5 and 6

	K'_{Glu} (mM)	$(K'_{Na})^{1/2}$ (mM)
Control	3.9 ± 0.4	27 ± 3
$+K_i^+$	2.0 ± 0.3**	38 ± 3**

**$P < 0.04$.

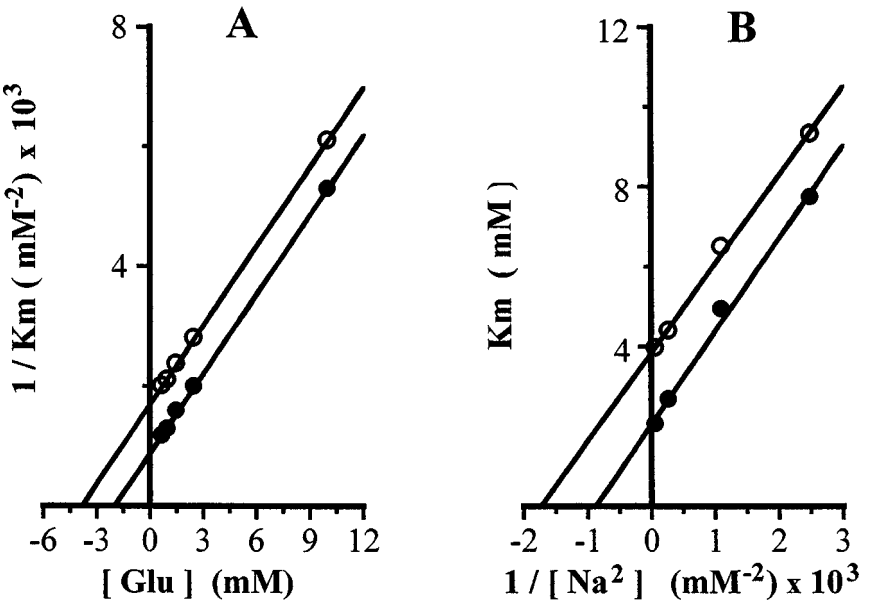

Fig. 7. Replot of data of Figs. 5 and 6. (A) K_m as calculated from the experimental slopes in Figs. 5(B) and 6(B) replotted as $1/K_m$ versus [Glu]. (B) K_m as calculated from the experimental slopes in Figs. 5(A) and 6(A) are replotted as K_m versus $1/[Na^+]^2$, (●) in the presence and (○) in the absence of potassium.

by Prezioso *et al.* (1996) and later confirmed by others (Sugawara *et al.*, 1998):

$$
\begin{aligned}
(C)_e + 2\,Na &\leftrightarrows (C\text{-}Na_2)_e & K_1 \cdot K_2 &= K_{Na}\\
(C\text{-}Na_2)_e + Glu &\leftrightarrows (Glu\text{-}C\text{-}Na_2)_e & K_{Glu}&\\
(Glu\text{-}C\text{-}Na_2)_e &\leftrightarrows (Glu\text{-}C\text{-}Na_2)_i & k_2&\\
(Glu\text{-}C\text{-}Na_2)_i &\leftrightarrows (C)_i + Glu + 2\,Na &&\\
(C)_i &\leftrightarrows (C)_e & k_3&
\end{aligned}
$$

This is best illustrated by the scheme in Fig. 8 (where * indicates the potassium binding site). In this scheme, K_{Na} is the product of the two sodium–carrier dissociation constant K_1 and K_2; K_{Glu} is the dissociation constant of the glutamate–carrier complex; k_2 and k_3 are the kinetic constants of the two rearrangement steps of the "charged" and "empty" carrier, respectively. The following assumptions are made: (i) The fast steps of the process, i.e., the interactions of the carrier with the substrates, are in rapid equilibrium and are then ruled by their equilibrium constants K_{Na} and K_{Glu}. (ii) The two rearrangement steps are rate determining and thus, in the stationary state, are ruled by their kinetic constants k_2 and k_3, which are assumed to be the same for forward and reverse directions.

On the basis of the assumptions made, Eq. (1) is obtained by defining the glutamate uptake rate $v = k_2$ $(Glu\text{-}C\text{-}Na_2)_e$, because we actually measure the rate of the step to which k_2 is referred, and considering that v is constant as long as $(Glu\text{-}C\text{-}Na_2)_e$ is stationary, and furthermore defining:

$$
V'_M = \frac{k_2 \cdot C_T}{1 + (k_2/k_3)} \qquad K'_{Glu} = \frac{K_{Glu}}{1 + (k_2/k_3)}
$$
$$
K'_{Na} = 2 \cdot K_{Na} \tag{4}
$$

where C_T is the total amount of carrier/mg protein.

Equations (2) and (3) have been used to fit the experimental kinetic data, both in the presence and in the absence of potassium. When K^+ is present inside the vesicles at a saturating concentration, we can assume that all the potassium binding sites are occupied by the cation. The experimental data presented show that Eq. (1) is still valid under these conditions, but something has changed in the values of its parameters. If we refer to the parameters defined in Eqs. (4), we can try to explain the data in Figs. 3, 5, 6, and 7 and Table II. The fact that V'_M is increased and K'_{Glu} is decreased in the presence of potassium can only be explained by an increase in the kinetic constant k_2, even though a simultaneous variation of k_3 and a decrease in K_{Glu}, the true dissociation constant of the carrier with respect to glutamate, cannot be excluded. The increase in K'_{Na} is bound to an increase in K_{Na}. As the binding of potassium modifies the dissociation constants of the carrier with respect to its substrates, it is necessary to define new dissociation constants for the potassium bound

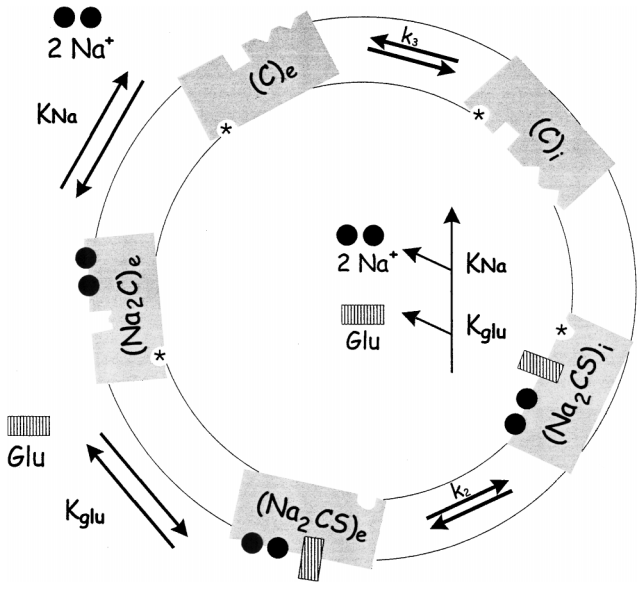

Fig. 8. Scheme of the sodium-glutamate cotransport across BBMV. *Indicates the potassium binding site. S stays for Glu.

carrier with respect to sodium and glutamate, that are indicated as $K_{Na,P}$ and $K_{Glu,P}$, respectively. Hence, when the activator is present, the substrates will be at rapid equilibrium with both the potassium bound and the potassium unbound forms of the carrier, and so four dissociation constants, K_{Glu}, K_{Na}, $K_{Glu,P}$, and $K_{Na,P}$ must be considered simultaneously.

It can be easily demonstrated that a sort of Haldane principle holds for the interaction of substrates with the carrier in the presence of potassium. This means that, if the activator modifies the true dissociation constants from K_{Glu} and K_{Na} to $K_{Glu,P}$ and $K_{Na,P}$, they must obey the relationship: $K_{Na} \cdot K_{Glu} = K_{Na,P} \cdot K_{Glu,P}$. On the other hand, from experimental data in Table II, it results that the product $K'_{Na} \cdot K'_{Glu}$ is unvaried in the presence of potassium. Such a result is compatible with the Haldane relationship only if the ratio k_2/k_3 does not change, i.e., if k_2 and k_3 are equally increased (see Eqs. (4)).

On the basis of these results, the presence of the K^+ cation would modify the kinetic mechanism of the transport process by introducing an "activated" form of the (Glu-C-Na_2)$_e$ complex and of the free carrier (C)$_e$, say (Glu-C_P-Na_2)$_e$ and (C_P)$_e$, that will rearrange to (Glu-C_P-Na_2)$_i$ and (C_P)$_i$ by kinetic constants $k_{2P} > k_2$ and $k_{3P} > k_3$. The increase in the two kinetic constants would be the same, and can be evaluated from Table I as the increase in $V_{M,Glu}$ in the presence of potassium, according to Eq. (4).

At nonsaturating concentrations of K^+, both potassium free and potassium bound forms will be present, and then the transport rate will be

$$v = k_2(\text{Glu-C-Na}_2)_e + k_{2P}(\text{Glu-C}_P\text{-Na}_2)_e$$

A rate equation can be achieved by the same procedure used for Eq. (1); by expressing v as a function of potassium concentration, and using auxiliary functions F, F_P, G, and G_P in order to simplify the expression, we have

$$v = C_T \cdot \frac{k_3[\text{Na}]^2[\text{Glu}]}{F_P + k_3 G_P} \times \frac{k_2 K_P + k_{2P}[\text{K}^+]}{(F + k_3 G/F_P + k_3 G_P)K_P + [\text{K}^+]} \qquad (5)$$

with $F = k_2[\text{Na}]^2[\text{Glu}] + k_3 K_{Na} K_{Glu}$; $F_P = k_{2P}[\text{Na}]^2[\text{Glu}] + k_{3[P]} K_{[Na]} K_{Glu}$; $G = [\text{Na}]^2[\text{Glu}] + K_{Glu}[\text{Na}]^2 + K_{Na} K_{[Glu]}$; $G_P = [\text{Na}]^2[\text{Glu}] + K_{Glu,P}[\text{Na}]^2 + K_{Na,P} K_{Glu,P}$ where K_P is the dissociation constant of the carrier–potassium complexes with respect to potassium. It is clear that Eq. (5) reduces to (1) when $[\text{K}^+] = 0$, while with $[\text{K}^+] \to \infty$ (saturating) it becomes similar to (1), but k_2 and k_3 are substituted by k_{2P} and k_{3P}. In general, such an equation is the analytical expression of plots like those in Fig. 2, and allows us to explain the variations of V_M and $K_{0.5}$ for potassium as a function of glutamate and sodium concentrations. In fact the data shown in Fig. 2 (both experimental sets), fitted in Eq. (5), well satisfy this analytical expression.

On the basis of these considerations, an attempt can be made to explain the effect of K^+ binding to the carrier at the molecular level. The parallel increases of k_2 and

k_3 probably means that the "movement" of the carrier is generally made easier by the cation binding, so enhancing the velocity of the rate determining steps of the transport process. In addition, the affinity for the first substrates, the two sodium cations, is reduced and that for glutamate is enhanced, according to the Haldane principle.

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