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# Electrogenicity of sodium/L-glutamate cotransport in rabbit renal brush-border membranes: a reevaluation

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**In order to clarify contradictory reports on the electrogenicity of sodium/L-glutamate cotransport, this cotransport was studied using brush-border membrane vesicles isolated from rabbit renal cortex. Beforehand, the claim that the symport of L-glutamate with $Na^+$ is linked to simultaneous antiport with $K^+$ has been confirmed by the demonstration that equilibrium exchange of L-glutamate is inhibited by potassium. Concerning the electrogenicity of the system, the following results are reported: net uptake of sodium-dependent L-glutamate uptake was stimulated when the transmembranal electrical potential difference was increased by replacing a sodium sulfate gradient by a sodium nitrate gradient. At 100 mM $Na^+$ the 'relative electrogenicity' of the initial uptake in the presence of intravesicular potassium was 2-times higher than in its absence. At a sodium concentration of 20 mM, when overall uptake was reduced, the relative electrogenicity in the presence of $K^+$ was even 3-fold higher than in $K^+$-free media. The relative electrogenicity of sodium/D-glucose cotransport measured under the same experimental conditions was not affected by $K^+$. These results are discussed in terms of a model where the apparent electrogenicity of a cotransport system is dependent on the extent to which the charge translocating step is rate limiting ('rate limitancy'). It is proposed that potassium antiport, while decreasing charge stoichiometry of $Na^+$/glutamate transport, increases the relative rate limitancy of the transport step translocating three cations (probably two $Na^+$, one $H^+$) together with one glutamate. Thereby the positive electrogenicity of glutamate uptake increases, in complete contrast to what would be expected from simple considerations of charge stoichiometry.**

## Introduction

L-Glutamate has been shown to be transported into renal brush border vesicles by $Na^+$-linked cotransport [2,3]. The presence of sodium appears to be absolutely required for L-glutamate translocation, and kinetic investigations have suggested that the stoichiometry of the transport system ($Na^+$/L-glutamate) is higher than unity [4,5].

Intravesicular potassium stimulates sodium/L-glutamate cotransport and a potassium gradient ($K_i^+ > K_o^+$) can support the intravesicular accumulation of L-glutamate [6]. The latter energetic coupling suggests a tight linkage between $Na^+$/L-glutamate symport and $K^+$ antiport [4,6,7].

Results on the electrogenicity of the transport system are contradictory. Some authors claim that the transport in the presence of $Na^+$ and $K^+$ is electroneutral (consistent with a charge stoichiom-

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Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

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etry of two $Na^+$/one $K^+$/one glutamate) [6] or positive electrogenic (consistent with a charge stoichiometry of three $Na^+$ : one $K^+$ : one glutamate) [3,4]. The most interesting findings in this context were reported by Burckhardt et al. [3] who observed only a very small positive electrogenicity of the $Na^+$/glutamate cotransport in the absence of $K^+$; positive electrogenicity increased, however, markedly when $K^+$ antiport occurred. At face value this observation seems to be a paradox as an outward movement of $K^+$ should decrease the 'charge stoichiometry' of the system and hence abolish or reduce positive electrogenicity.

We, therefore, reinvestigated the phenomenon of the electrogenicity and its unmasking by potassium. In the following it is proposed that potassium renders L-glutamate uptake electrogenic by increasing the 'relative rate limitancy' of that transport step in which net positive charge translocation (i.e., translocation of two or three $Na^+$ and one glutamate) occurs. Thereby an increase in electrogenicity in the presence of $K^+$ can easily be explained. The contradictions in previous reports regarding the apparent electrogenicity of L-glutamate transport are thus probably due to differences in rate limitancy of the charge-translocating transport steps under the different experimental conditions employed.

Some of the results have been presented in preliminary form [1].

## Methods

*Membrane preparation.* Brush border membrane vesicles were isolated from young adult male New Zealand White rabbit kidney cortex by a magnesium precipitation method as described originally by Booth and Kenny [8] and modified by Aronson [9]. In the final membrane fraction, the brush border membrane marker enzymes alkaline phosphatase and aminopeptidase M were enriched 9–12-fold, and the activity of the basal-lateral marker ($Na^+ + K^+$)-ATPase was reduced as compared to the original homogenate. Final pellets were resuspended in vesicle buffer. For tracer exchange experiments the buffer contained 25 mM NaCl/20 mM Hepes/0.1 mM glutamic acid/the desired amount of KCl or choline chloride/*N*-methylglucamine chloride to bring osmolarity to 300 mosM, and was titrated to pH 7.4 with HCl. For determination of the initial uptake rate the vesicle buffer contained 135 mM *N*-methylglucamine chloride/20 mM Hepes/10 mM $K_2SO_4$ or choline sulfate. The pH was adjusted to 7.4 with $H_2SO_4$. Protein concentrations of these suspensions were 5–10 mg/ml. Vesicles were stored at −70 °C. Prior to use, vesicles were rapidly thawed in a 37 °C water bath, homogenized five times through a 23 gauge needle, and left to stand at room temperature for 20 min.

*Tracer exchange.* Equilibrium exchange was performed at 25 °C. Incubation media consisted of the appropriate vesicle buffer to which 5 μCi L-[$^3$H]glutamic acid (36 Ci/mmol) was added. Incubation was initiated by adding 20 μl vesicles to 130 μl incubation medium. Exchange was stopped by adding 20 μl reaction mixture to 1 ml of an ice-cold stop solution containing 100 mM mannitol/20 mM Hepes-Tris (pH 7.4)/150 mM NaCl. The stop solution – containing the reaction mixture – was then immediately transferred with a Pasteur pipette onto a Millipore filter (0.45 μm pore size) kept under suction. The filter was washed once with 3.5 ml ice-cold stop solution. The radioactivity remaining on the filters was analyzed by standard liquid scintillation counting techniques. All media and solutions used for isolation and transport of vesicles were filtered through a Millipore filter (0.22 μm pore size) immediately before use to avoid bacterial contamination.

*Initial uptake rate.* The initial uptake rate was determined by mixing 10 μl of membrane suspension kept at 25 °C with 85 μl incubation medium kept at the same temperature. The incubation medium contained 100 mM sodium either as nitrate or sulfate salt/20 mM Hepes/75 mM *N*-methylglucamine chloride/0.1 mM L-glutamate or D-glucose. The pH of the incubation medium was adjusted to 7.4 with $H_2SO_4$. 5 μCi of tritiated substrate were used per vial. Uptake was terminated after 2, 6, 10 or 14 s of incubation at 25 °C by the addition of 1 ml ice-cold stop solution. Rapid filtration followed as described above. The four time points were fitted to a linear regression line, $r^2$ was always higher than 0.998.

It should be mentioned that the (mostly) straight line between these time points, if extrapolated,

does not pass the origin. This deviation from linearity before the first 2 s could be attributed to the presence of very small vesicles which would fill more rapidly than the bulk of the larger ones, or to the rapid binding of glutamate to unspecific sites at the vesicle surface, or to both. We did not consider these deviations to be crucial in the present context, as similar (though more scattered) results to our present ones are obtained when either the intercept or the 2- or 6-s uptake values were used for the initial rate instead of the slope of the linear part.

*Protein and enzyme assays.* Protein was determined after membrane precipitation with ice-cold trichloroacetic acid (10%) by a modified Lowry procedure with bovine serum albumin as a standard [10]. Alkaline phosphatase (EC 3.1.3.1) and $(Na^+ + K^+)$-stimulated ATPase (EC 3.6.1.3) were determined as described by Berner and Kinne [11].

*Materials.* L-[$^3$H]Glutamic acid and D-[$^3$H]glucose were purchased from Amersham (Buchler, Braunschweig, F.R.G.). All other chemicals used were obtained through Sigma (Taufkirchen, F.R.G.) and were of at least analytical grade quality.

*Statistics.* For statistical evaluation paired or unpaired Student *t*-tests were applied. *p* values of less than 0.05 were considered statistically significant.

## Results

### *Effect of $K^+$ on L-glutamate transport*

*Equilibrium exchange.* In order to provide evidence that under the experimental conditions employed in this study $Na^+$/glutamate symport can be coupled to $K^+$ antiport, we studied tracer exchange experiments under equilibrium conditions for sodium, potassium, and (unlabelled) glutamate. As shown in Fig. 1, L-glutamate exchange decreases with increasing $[K^+]$.

In the range of $K^+$ concentrations tested, the inhibition is consistent with an interaction of $K^+$ with the transporter probably with a stoichiometry of one as determined by Fukuhara and Turner [12]. As elaborated in more detail in the Discussion, inhibition of tracer exchange provides strong evidence for the occurrence of $K^+$ antiport linked with the $Na^+$/glutamate symport.

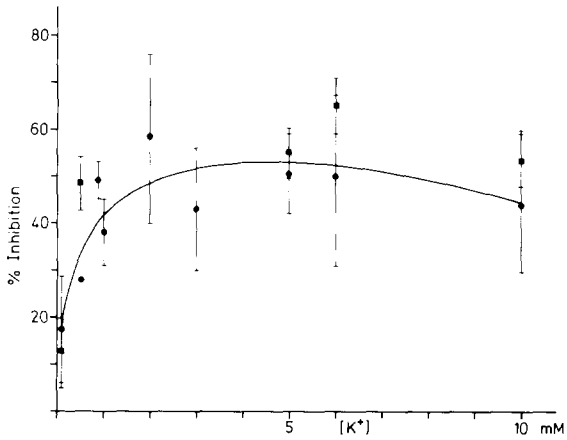


Fig. 1. Inhibition of glutamate equilibrium exchange by $K^+$. Vesicles were preloaded with 25 mM NaCl/20 mM Hepes-Tris (pH 7.4)/115 mM *N*-methylglucamine chloride/0.1 mM L-glutamic acid/and various concentrations of KCl (■). In some experiments (●) choline chloride was used to substitute for KCl. The total concentration of $K^+$ + choline was kept constant at 20 mM. Incubation media were identical, but contained in addition 5 $\mu$Ci L-[$^3$H]glutamic acid. Equilibrium values (glutamic acid uptake determined after 120 min) were $125.5 \pm 6.6$ pmol/mg protein for choline-containing vesicles and $130.8 \pm 15.3$ pmol/mg protein for $K^+$-containing vesicles. The ordinate shows the percentage of inhibition, the abscissa the $K^+$ concentration, which is equal inside and outside the vesicle. Each point of the curve represents the average of six independent measurements with the standard deviation indicated.

*Initial uptake rate.* In Fig. 2 the effect of intravesicular $K^+$ on the initial uptake rate of L-glutamate is shown. In the presence of a $Na_2SO_4$ gradient potassium stimulates L-glutamate uptake by about 100%, in the presence of nitrate by about 200%. By contrast, D-glucose uptake which was simultaneously studied in the same preparation is not affected by $K^+$. Hence, membrane potential changes, which might have been induced by the $K^+$ gradient and could have stimulated L-glutamate uptake, can be excluded.

*Effect on electrogenicity.* In order to investigate the effect of potassium on the electrogenicity of glutamate transport in more quantitative terms we introduced as a measure of electrogenicity the relative electrogenicity. This relative electrogenicity was defined as the differential of the overall flux to the corresponding electrical activity coefficient ($\epsilon = \exp(-F\Delta\psi/2RT)$) relative to the un-

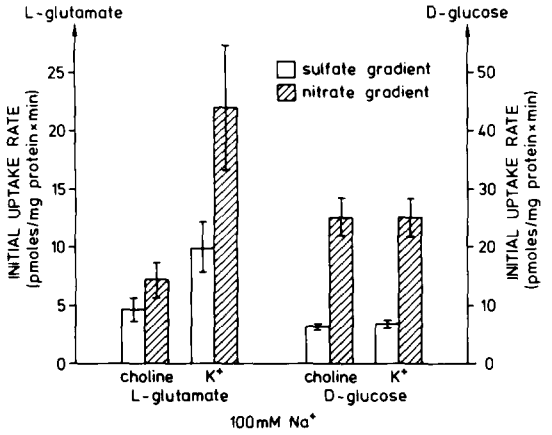


Fig. 2. Effect of the electrical potential on the initial uptake rate of glutamate and glucose, respectively, in the absence and presence of intravesicular $K^+$. The electrical potential was changed by replacement of the sulphate gradient by a nitrate gradient as described under Methods. Each column represents the average of 11 independent samples. For glutamate the difference between $NO_3$ and $SO_4$ is not significant without potassium, but significant ($P < 0.05$) with potassium. For glucose the corresponding differences are highly significant, but there is no significant difference in the presence or absence of $K^+$.

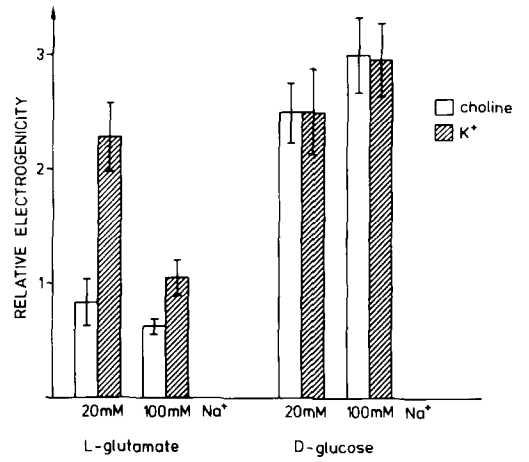


Fig. 3. Effect of changes in extravesicular $Na^+$ concentration on the relative electrogenicity of glutamate and glucose, respectively. The relative electrogenicity is defined and evaluated as described in the text. In the experiments at 20 mM $Na^+$ concentration 80 mM of the sodium chloride were replaced by choline chloride. Each column represents the average of ten independent experiments, with the standard error indicated. At low outside $Na^+$ the relative electrogenicity rises, especially in the presence of intravesicular $K^+$, where the difference is highly significant ($P < 0.01$). By contrast, the relative electrogenicity of glucose decreases with lowering outside $Na^+$ but not significantly.

changed flux $J_a$. For practical reasons we used the corresponding differences as follows:

$$\frac{J_2 - J_1}{\epsilon_2 - \epsilon_1} \cdot \frac{1}{J_1}$$

In this equation $J_2$ and $J_1$ represent the flux observed in the presence of the nitrate or sulfate gradient, respectively. The difference between $\epsilon_2$ and $\epsilon_1$ was considered to be constant and set at unity.

Fig. 2 shows that replacement of the sulfate gradient by a nitrate gradient, a manoeuvre which, as evidenced by the stimulation of the sodium/D-glucose cotransport, renders the vesicle inside more negative, stimulates L-glutamate uptake in the presence as well as in the absence of $K^+$. However, in the presence of potassium both the degree of stimulation (160% compared to 220%) as well as the relative electrogenicity (0.6 compared to 1.2) are significantly higher (Fig. 3).

These data demonstrate that L-glutamate uptake shows an increased positive electrogenicity under circumstances where from simply considering charge stoichiometry [13] a decrease in electrogenicity would have been expected.

*A model*

In order to explain this apparently paradoxical behaviour of the electrogenicity we developed a model which is based on the assumption that electrogenicity manifests itself only to the extent that a charge-translocating step limits the overall transport rate (rate limitancy). This model is illustrated in Fig. 4. In the absence of $K^+$ the rate of translocation of the charge-translocating carrier substrate complex is assumed to be considerably faster than the electroneutral relocation of the empty carrier. $K^+$ by binding to the substrate-free carrier is supposed to accelerate the relocation step to such an extent that its rate markedly exceeds that of the charge-translocating carrier substrate complex. The resulting increase in rate limitancy of the latter transport step makes the electrogenicity manifest.

If this explanation is correct, a similar change in electrogenicity should be expected after an analogous shift in rate limitancy by means other than by the addition of *trans*-$K^+$. Provided that the substrate-translocating step is potential- de-

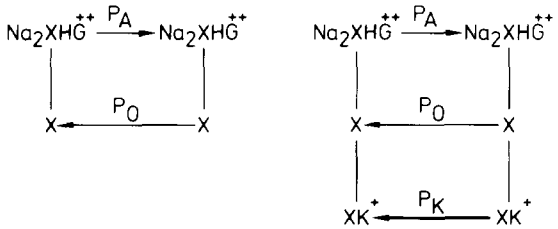


Fig. 4. Simplified model of $Na^+$-linked glutamate transport without $K^+$ inside (left) and with $K^+$ inside (right). $P_A$, $P_0$, and $P_K$ are the rate coefficient (probability coefficient) for the fully loaded, the empty, and the $K^+$-loaded carrier species, respectively. The translocator (X) is assumed to be neutral.

pendent, slowing it down should also increase the electrogenicity of the overall process. This has been tested experimentally by reducing the $Na^+$ concentration on the *cis* side. As shown in Fig. 3 and Table I the relative electrogenicity of L-glutamate transport increases when the sodium concentration in the incubation medium is lowered from 100 mM $Na^+$ to 20 mM $Na^+$. This increase is more pronounced in the presence of $K^+$ than in the absence of $K^+$. In contrast, the relative electrogenicity of D-glucose uptake under the same conditions tends to decrease rather than to increase. This result could indicate that in the D-glucose sodium cotransport it is an electroneutral step, namely the sodium-linked glucose translocation, which becomes more rate limiting under these experimental conditions.

In order to demonstrate that the manifestation of electrogenicity of L-glutamate transport is due to a shift in rate limitancy from the (electroneutral) return reaction to the potential-dependent, substrate-translocating forward reaction a semi-quantitative estimate of this rate limitancy has been applied. We have attempted such an estimate based on the following considerations. In a system of several flows in series, each of these flows may add to the overall limitation; the slower such flow, the greater its limiting effect on the overall flow. As treated in more detail elsewhere (Heinz, E., unpublished results), one may accordingly derive the 'fractional rate limitancy' for each of these flows, the sum of which limits the overall flow.

A system of mediated transport can be considered such a system, consisting of at least four flows in series: two for loading and unloading the translocator at the two interfaces, and the two translocations through the barrier of the substrate-loaded and substrate-unloaded translocator species, respectively. Assuming quasi equilibrium at the interfaces, there remain only the translocation steps to limit significantly the overall transport rate. Each of these flows is a function of the probability that the translocator species concerned jumps the barrier per time unit ($P_i$) times the difference in electrochemical activity of this species between the two interfaces. The rate limitancy of any translocation step in such a system can, in analogy to an equivalent circuit, be defined as the ratio of the overall transport rate over the corresponding one under the conditions that the probabilities of the other step(s) in series are infinitely greater than that of the step under consideration. Accordingly, the rate limitancy of the

TABLE I

EFFECT OF POTASSIUM,SODIUM, AND THE MEMBRANE POTENTIAL ON L-GLUTAMATE (A) AND D-GLUCOSE (B) UPTAKE BY RABBIT KIDNEY BRUSH BORDER MEMBRANES

Data are given as the slope of the linear regression lines obtained from four time points. They represent mean values derived from *n* experiments with their standard deviation.

| | Choline | | | | Potassium | | | |
|---|---|---|---|---|---|---|---|---|
| | $SO_4$ | *n* | $NO_3$ | *n* | $SO_4$ | *n* | $NO_3$ | *n* |
| (A) | | | | | | | | |
| 20 mM sodium | 1.76 ± 0.36 | 11 | 3.00 ± 0.49 | 11 | 2.52 ± 0.33 | 11 | 7.83 ± 0.89 | 11 |
| 100 mM sodium | 4.65 ± 1.03 | 11 | 7.35 ± 1.54 | 11 | 9.93 ± 2.23 | 11 | 22.0 ± 5.38 | 10 |
| (B) | | | | | | | | |
| 20 mM sodium | 3.16 ± 0.29 | 11 | 10.87 ± 1.02 | 11 | 3.02 ± 0.25 | 11 | 10.32 ± 0.92 | 11 |
| 100 mM sodium | 6.19 ± 0.49 | 11 | 25.29 ± 3.09 | 11 | 6.82 ± 0.61 | 11 | 26.63 ± 3.02 | 11 |

glutamate-translocating (forward) step would be obtained by dividing the overall transport rate by that under the condition that the probability of the return steps, i.e., providing the empty and/or $K^+$-loaded translocator is infinite. Since the latter condition cannot be verified experimentally in our system, we may resort to a crude approximation which is obtained, as shown by Heinz (unpublished results) by testing the relative response of the overall transport rate of a change in the substrate concentration, e.g., to a change in *cis*-$Na^+$. Although the value thus obtained, called by us the 'relative rate limitancy', is not identical with the true fractional rate limitancy, it should be in the same direction and approximatley proportional to the latter (provided that the substrate concentration does not exceed too much the effective dissociation constant).

For practical purpose we used again the corresponding differences

$$\frac{J_2 - J_1}{A_2 - A_1} \cdot \frac{A_1}{J_1}$$

where $J_2$ and $J_1$ are the fluxes observed in the presence of $A_2$ (100 mM sodium) or $A_1$ (20 mM sodium), respectively.

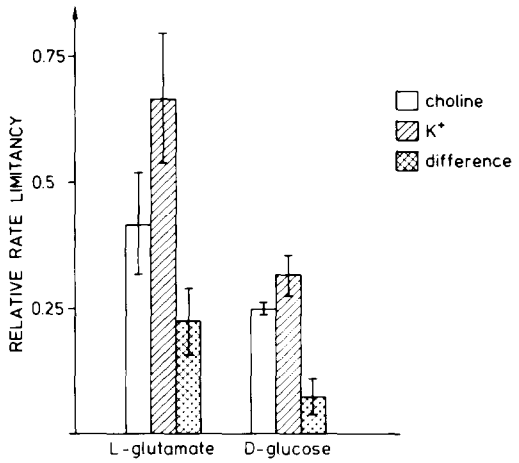


Fig. 5. The relative rate limitancy of L-glutamate and D-glucose, respectively, in the presence and absence of intravesicular $K^+$, respectively. Experimental conditions were as in Fig. 3. Each column represents the average of ten independent experiments with the standard error indicated. The relative rate limitancy was obtained as described in the text. The difference between the values for L-glutamate in the presence and absence of $K^+$ are highly significant with paired comparison ($P < 0.01$). The corresponding differences for D-glucose are not significant.

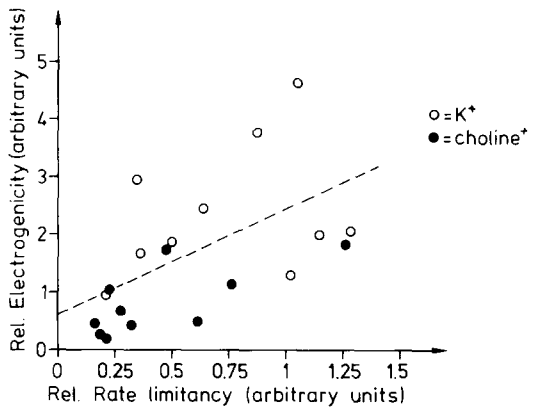


Fig. 6. The relative electrogenicity is plotted versus the corresponding rate limitancy of altogether 20 independent experimental determinations. The dotted line is drawn by the method of least-squares. Its slope is significantly different from 0 ($P = 0.01$).

It is evident from Fig. 5 that in the presence of potassium the rate limitancy for $Na^+$/L-glutamate translocation is significantly higher than in its absence. As expected, the rate limitancy for $Na^+$/D-glucose translocation is not affected by the presence of potassium. Thus, for L-glutamate transport an increased rate limitancy is associated with an increased relative electrogenicity.

This positive correlation between relative electrogenicity and rate limitancy is also evident from Fig. 6, where the individual experimental results of this study have been plotted. The exact correlation between these two parameters remains to be established.

## Discussion

*General aspects*

In a qualitative aspect the studies reported above corroborate the findings of both Schneider et al. [3] and Burckhardt et al. [4], namely that sodium/glutamate cotransport in the absence of intravesicular $K^+$ is barely potential sensitive. In addition, we could confirm the observation by Burckhardt et al. [4] and Koepsell et al. [5] that, in the presence of $K^+$, the uptake of L-glutamate becomes strongly potential sensitive. Furthermore, evidence for antiport of $K^+$ was obtained in tracer

exchange studies, strengthening the argument derived from studies on the energetic coupling of potassium gradients to $Na^+$/glutamate symport [6]. The inhibition of $Na^+$/glutamate exchange by potassium can be explained by the assumption that, due to the formation of the $K^+$-carrier complex, a major fraction of the translocator becomes unavailable for equilibrium exchange through the glutamate-sodium carrier pathway. It should be noted that even at the highest potassium concentration no complete inhibition is observed. We attribute this partial inhibition to the fact that potassium at a high concentration can undergo cycloport as observed and reported with citrate transport [14].

*Relative electrogenicity and relative rate limitancy*

In the results presented above a difference rather than the differential has been used to quantify the relative electrogenicity or relative rate limitancy of glutamate and glucose transport. This was necessary because of the scattering of the data. The results are therefore only approximate but should nonetheless give the correct direction of the induced changes. In addition, in order to be able to compare the values for relative electrogenicity in the current study, the potential was assumed to be the same whether it was generated in the absence or presence of $K^+$. This appears to be the case, since electrogenic sodium-dependent D-glucose uptake, though being electrogenic, is independent of potassium under all conditions studied. Furthermore, glutamate transport itself does not seem to alter the membrane potential significantly as a high concentration of L-glutamate does not affect sodium/D-glucose uptake (data not shown).

*The model*

As detailed in the Appendix a relative simple model has been used to describe L-glutamate transport.

As computer calculations showed that even the simplified model yields results which are qualitatively similar to the experimental results, its use seems to be justified.

On the basis of this model electrogenicity should presumably be unmasked by any means that shift the 'rate limitancy' from an electroneutral step to a charge translocating one, for instance by accelerating the former and/or by retarding the latter. This is most obvious with a system in which an electropositive substrate ($A^{2+}$) is translocated by an electroneutral carrier (X). It is seen that in the equation for the initial rate the electrical term ($\epsilon^2$) appears both in the numerator and in the denominator. Hence, electrogenicity may be enhanced in different ways, for instance, by increasing the rate of X or by decreasing that of $XA^{2+}$. In the case of glutamate the former might result if the back reaction of the substrate-free translocator is accelerated by binding of $K^+$. Evidence for a binding of $K^+$ to the transporter can be derived from the decrease of glutamate equilibrium exchange in the presence of $K^+$ which is most easily explained by the formation of $XK^+$ complexes which are no longer available for exchange. A decreased rate of $XA^{2+}$ translocation was achieved by decreasing the sodium concentration. Thus, so far all experimental observations with L-glutamate are consistent with the proposed model. Before drawing a conclusion, however, alternative hypotheses regarding the $K^+$-induced electrogenicity of the $Na^+$/glutamate cotransport should be considered.

(1) $K^+$ could increase the charge stoichiometry, e.g., by increasing the number of $Na^+$ or $H^+$ ions translocated together with glutamate. Since at least two more cations are required to compensate for the reduction of charge stoichiometry induced by the binding of one $K^+$ to the transporter this possibility seems to be unlikely, also in view of the difficulty in plausibly explaining the mechanism underlying such a conformational change.

(2) $K^+$ at the inside of the vesicle could, as $H^+$ does at the outside, convert the carrier to a channel [15]. Such an action would, however, not explain the inhibition of equilibrium exchange by $K^+$, nor would it be compatible with the 'active accumulation' of glutamate observed in the presence of an outwardly directed $K^+$ gradient [6].

(3) $K^+$ could increase the rate coefficient for the release of $Na^+$-glutamate on the inside of the vesicle. Such an explanation would imply that the off reaction (i.e., dissociation of sodium and glutamate from the carrier) is rate limiting for the overall transport, and at the same time is potential dependent. The first implication is unlikely in view of the generally assumed rapidity of 'off'

reactions as compared to the transport rate. Moreover, such an effect, if real, would only account for the *trans*-stimulation of glutamate transport by $K^+$ but not for a change in electrogenicity, which should be the same with and without $K^+$.

It is also interesting to discuss why the electrogenic sodium/glucose cotransport behaves different from the sodium/glutamate cotransport, in particular in those experiments where different sodium concentrations were used. Under these conditions relative electrogenicity of the D-glucose transport tended to decrease. Such a behaviour would be consistent with the assumption that the charge-translocating step and the rate-limiting step would be the relocation of a negatively charged empty carrier rather than the translocation of the sodium/glucose carrier complex. That the empty carrier of the sodium/D-glucose cotransporter might indeed be negatively charged is suggested by the observation that an inside negative membrane potential increases the number of D-glucose cotransporter available at the outer surface of the membrane [9]. Furthermore, biochemical studies using isoelectric focussing of membrane extracts revealed a $pK_I$ of 5.4 for the transporter when studied in the presence of neutral detergents [16]. That the rate coefficient of the empty carrier is smaller than that of the substrate-loaded one can also be concluded from transstimulation experiments which showed that D-glucose tracer influx is stimulated by the presence of D-glucose inside the vesicles (negative tracer coupling).

The above-stated relation between relative electrogenicity and rate limitancy, which explains the apparently paradox effect of $K^+$ on sodium/glutamate cotransport, has implications for experiments in which the charge stoichiometry of a transport system is determined on the basis of its potential sensitivity. In extreme cases the relative electrogenicity may be entirely different from the charge stoichiometry.

For glutamate the relative electrogenicity at 20 mM sodium approaches the electrogenicity of the sodium/glucose cotransport system. From direct and indirect measurements of stoichiometry it can relatively safely be assumed that the charge stoichiometry for the latter transport system is unity [13]. Thus, for the translocation of glutamate together with $Na^+$ (and $H^+$) in exchange with $K^+$ also a charge stoichiometry of one can be assumed. Since probably only one $K^+$ is involved in the interaction with the carrier system and at pH 7.4 glutamic acid is almost completely dissociated into glutamate, three positive charges, most probably two sodium ions and one $H^+$, are symported with glutamate. This charge stoichiometry is in close agreement with that of a transport model proposed recently on the basis of kinetic data on the sodium and pH dependence of glutamate transport by Nelson et al. [7]. On the other hand, though, there are some major differences between the last-mentioned model and our model (Fig. 4), especially with respect to the function of hydrogen ions.

## Appendix

For the model depicted in Fig. 4, the overall rate equation for the presence and absence of *trans*-$K^+$ have been derived under the following simplifying assumptions:

(i) The initial rate is 'ture', i.e., glucose and $Na^+$ are only on the *cis* side ($'$), $K^+$, if present, only on the *trans* side ($''$).

(2) The system is in 'quasi equilibrium', i.e., the interactions between translocator and ligands are very fast as compared to the translocation through the carrier.

(3) Only the fully loaded translocator ($XGNa_2H^{2+}$), the empty one (X) and the $K^+$ loaded one, ($XK^+$) if present, are significantly translocated. The existence and movement of partially loaded carriers have been neglected. Accordingly we treated the 'transportate', i.e., the product of the individual ligands $[Glu]\cdot[Na^+]^2\cdot[H^+]$ as a single ligand, (A).

(4) The binding of $K^+$ and that of glutamate are mutually exclusive (high negative cooperativity).

(5) The system is symmetrical.

*Equations*
with $K^+$

$$J_a = X_T \frac{P_a(P_0 + P_K\gamma''\epsilon^{-1})\alpha'\epsilon^2}{(1+\alpha')(P_0 + P_K\gamma''\epsilon^{-1}) + (1+\gamma'')(P_a\alpha\epsilon^2 + P_0)} \qquad \text{(A-1)}$$

without $K^+$

$$J_a = X_T \frac{P_a P_0 \alpha' \epsilon^2}{2P_0 + (P_0 + P_a \epsilon^2)\alpha'} \quad \text{(A-2)}$$

$J_a$ is the overall transport rate for glutamate, $X_T$ is the total number of translocator sites per unit of translocating membrane

$$\alpha' = \frac{r_a \cdot [G] \cdot [Na^+]^2 \cdot [H^+]}{K_G \cdot K_{Na}^2 \cdot K_H}$$

$K_G$, $K_{Na}$, $K_H$ are the intrinsic dissociation constants for glutamate (G), $Na^+$, $H^+$, respectively. $r_a$ is the overall cooperativity factor, i.e., the product of all possible cooperativity coefficients between the binding sites for glutamate, sodium ions and protons, respectively.

$$\gamma'' = \frac{[K^+]''}{K_K}$$

$K_K$ being the dissociation constant of $XK^+$, the translocator-$K^+$ complex. $\epsilon$ is the electrochemical activity coefficient (square root):

$$\epsilon = e^{-F\Delta\psi/2RT}$$

$F$ being the Faraday constant, $\Delta\psi$, the electrical potential difference $R$, $T$, gas constant and absolute temperature, respectively. $P_a$, $P_c$, $P_0$ are the rate coefficients for the fully loaded, $K^+$-loaded, and unloaded translocator, respectively.

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