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# Analyzing an Electrogenic Cotransporter

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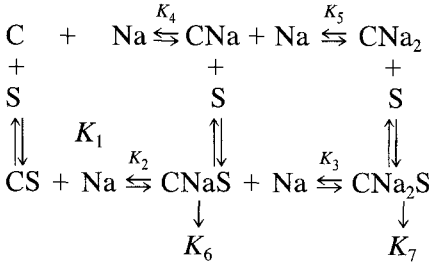
**Abstract.** A model for the sodium-dependent accumulation of glutamate by synaptosomes has been presented which fits the data of Wheeler and his coworkers and supports their hypothesis of an electrogenic cotransporter. Since their hypothesis was based on experimental data on the operation of the cotransporter on the outer membrane, the model was expanded to predict events when the cotransporter was operating on both sides of the membrane. The model predicts that the accumulation of glutamate is sensitive to the synaptosomal sodium and emphasizes the importance of the sodium/potassium pump to maintain this value. A model which uses only an electrogenic form of the cotransporter on the external membrane and a neutral form on the inside of the membrane predicts too much or too little accumulation of glutamate at different membrane potentials. A model which uses an electrogenic cotransporter on the external membrane and a concentration-dependent sodium glutamate leak would require a significant increase in the permeability of sodium glutamate when the membrane depolarizes. Only the operation of all four mentioned mechanisms will fit experimental data at two different external sodium concentrations and over the range of membrane potentials measured experimentally.

**Key words:** Cotransport — Electrogenic — Glutamate transport — Modeling

## Introduction

Wheeler and his associates (1979, 1983) had developed a set of equations which described the cotransport of glutamate and sodium across the membrane of synaptosomes from rat cortical tissue. The cotransporter had two forms: (i) one form transported one glutamate and one sodium as a neutral pair; (ii) a second form transported one glutamate and two sodium ions as a positively charged moiety and was electrogenic. After considering several alternatives, they arrived at a pathway which gave the best fit to the experimental data. This pathway is outlined below.




and the relevant equation for this pathway was

$$v = \frac{\dfrac{K_3K_6[\mathrm{Na}] + K_7[\mathrm{Na}]^2}{K_2K_3 + K_3[\mathrm{Na}] + [\mathrm{Na}]^2}\,[\mathrm{S}]}{\dfrac{K_1K_2K_3K_4K_5 + K_1K_2K_3K_5[\mathrm{Na}] + K_1K_2K_3[\mathrm{Na}]^2}{K_2K_3K_4K_5 + K_3K_4K_5[\mathrm{Na}] + K_4K_5[\mathrm{Na}]^2} + [\mathrm{S}]}$$

where $v$ is the initial velocity or flux of glutamate into the synaptosomes from a medium of S glutamate concentration and Na sodium concentration in mol/liter. The dimensions are nanomol/synaptosome from 10 mg cortical tissue/min. This value may be converted to mol/liter/min by dividing by 300 since Wheeler (1979) had shown that $0.30 \times 10^{-6}$ liter water were in the synaptosomes from 10 mg of cortical tissue. The symbols $K_1$ through $K_5$ are dissociation constants in mol/liter. Symbols $K_6$ and $K_7$ are maximal velocities or fluxes of the two forms of the cotransporters in nanomol/synaptosome in 10 mg

**Table.** Constants giving best fit to velocity data for uptake of glutamate by rat cortical synaptosomes.[a]

| | |
|---|---|
| $K_1 = 3.7132 \times 10^{-5}$ | $K_5 = 8.3615 \times 10^{-3}$ |
| $K_2 = 10.4481$ | $K_6 = 1.5827 \times 10^{3}$ |
| $K_3 = 4.3981 \times 10^{-5}$ | $K_7 = 2.6678$ |
| $K_4 = 0.33579$ | |

[a] From Wheeler and Graves (1983). $K_1$ through $K_5$, mol/liter. $K_6$ and $K_7$ nanomol/10 mg/min.

tissue/min. They are the product of the rate constant $k_6$ or $k_7$ and the saturated concentration of that particular form of cotransporter. Implicit in the derivation is the assumption that the binding and unbinding of ligand with cotransporter are very rapid and that the rate-limiting step is the transit of the cotransporter-ligand complex from one side of the membrane to the other. Experiments which measured initial uptake of glutamate as a function of glutamate concentration and sodium concentration in the medium were used to find the parameters which gave the best fit. The Table contains these parameters.

The authors also provided experimental evidence which demonstrated that synaptosomes could accumulate glutamate to values as high as 1,800 times the medium concentration of $10^{-5}$ M and that distribution ratio and initial velocity of uptake decreased linearly with a decrease in potential difference brought about by the addition of external potassium. They proposed that one form of the cotransporter was electrogenic and that the free energy for the accumulation of glutamate came from the sodium gradient and the membrane potential.

The accumulation of glutamate is a balance between the uptake from the medium and loss from the synaptosome. The model presented here was designed to identify those factors that were paramount in establishing the accumulation ratios reported experimentally by Wheeler and his colleagues. It considers four mechanisms which may be operating to produce the high distribution ratios:

(i) The cotransporter on the external side of the membrane has a neutral form and a positively charged form driven by the membrane potential.

(ii) An identical cotransporter is located on the inner side of the membrane with a finite value for the flux of the neutral form, but a value of 0 for the positively charged form. This arrangement will hold as long as the membrane potential is negative inside relative to ground outside. If the membrane potential should reverse, then the positively charged transporter on the inner side of the membrane would have a finite value and the positively charged transporter on the outer membrane would be set at 0.

(iii) Sodium glutamate moves down its concentration gradient.

(iv) A sodium/potassium pump keeps the synaptosome content of sodium constant by the extrusion of three sodiums and the gain of two potassium ions. For every exchange of three sodiums for two potassiums, one chloride is lost to maintain electrical neutrality.

## Materials and Methods

A program for the Macintosh computer was written in Microsoft Quick Basic to model the interaction among these four mechanisms. The program executed the following sequence of events:

(i) Initial conditions were entered for the medium concentrations of glutamate and sodium in mol/liter from experimental data. The synaptosomal concentration of sodium in mol/liter was set as an arbitrary value and a synaptosomal concentration of glutamate was set equal to 0. These data were followed by the entry of the parameter values listed in the Table for calculation of the glutamate flux velocity.

(ii) The program then solved for the velocity of flux into synaptosomes with both forms of the cotransporter in operation. This influx is designated as FL(2, 2).

(iii) It solved for the velocity of glutamate flux out of the synaptosome with both forms of the cotransporter in operation. This efflux is designated as FL(2, 1).

(iv) The net gain of glutamate, DELTA, was calculated as FL(2, 2) − FL(2, 1)*T, where T is the time interval over which the net flux occurred.

(v) The program then solved for the glutamate flux into the synaptosomes when only the neutral form was functioning. This value is designated as FL(1, 2).

(vi) It calculated the glutamate flux out of the synaptosomes when only the neutral form was functional. This value is designated as FL(1, 1).

(vii) The coupling ratio, CPLG, for sodium/glutamate was calculated as:

$$CPLG(I) = (FL(1, I) + (FL(2, I) - FL(1, I))*2)/FL(2, I)$$

where I = 1 for the inner side of the membrane and I = 2 for the external side of the membrane. * designates multiplication.

(viii) The net gain for sodium, DL was calculated as:

$$DL = (FL(2, 2)*CPLG(2) - F1(2, 1)*CPLG(1))*T$$

(ix) The exit of sodium glutamate down its concentration gradient was defined by a simple Fick diffusion equation.

$$FLUX = P(CO - C)$$

where FLUX is the flux of sodium glutamate in mol/liter min, P is the permeability coefficient in $min^{-1}$, CO is the external concentration of glutamate and C is the internal concentration of glutamate in mol/liter.

(x) The net change in synaptosomal glutamate was calculated as:

$$S = S + DELTA + FLUX*T$$

(xi) The net change in synaptosomal sodium was calculated as:

$$NA = NA + DL + (FLUX - PUMP)*T$$

where PUMP is the flux of sodium produced by the sodium/potassium pump necessary to keep synaptosomal sodium constant.

(xii) The effect of the membrane potential on the cotransporter was programmed by multiplying $K_7$, the flux of the positively charged form of the cotransporter, by a coefficient which was a linear function of the voltage. The coefficient was assigned a value of 1 at −84.2 mV. This membrane potential was the average value reported by Wheeler and Graves (1983) for synaptosomes in Krebs-Henseleit medium without glutamate present. At 0 mV, $K_7$ was set at 0. It is important to emphasize, however, that even though the positively charged form of the cotransporter did not function, it was still able to combine with one glutamate and two sodiums and commit that amount of the total available transporter to its pathway. It is also important to note that on the inner side of the membrane, the value of $K_7$ was set at 0 in order to model the difference in potential between the two sides of the membrane.

## Results

Figure 1 is a plot of initial velocity of glutamate uptake as a function of the sodium concentration in the medium as predicted by the constants listed in the Table. The plot was prepared to validate the computer program. At a glutamate concentration of $1 \times 10^{-5}$ M with $K_7$ at its specified value, the plot was identical with the graph shown in Fig. 1 of Wheeler (1979). The other three plots describe the behavior of the cotransporter when the potential difference is 0 and $K_7$ is 0. Under these circumstances, only the neutral form of the cotransporter is functional. Nevertheless, a fraction of the total transporter is still committed to the positively charged form depending upon the external sodium concentration. For example, at a concentration of glutamate of $1 \times 10^{-5}$ M, the velocity of the neutral cotransporter is maximal at 0.030 M sodium. At higher concentrations, the velocity decreases as more of the total transporter is committed to the positively charged form, whether the latter is functional or not.

The other important point to make in this figure is the effect of increasing the glutamate which is available for transport. Note that the curves at $50 \times 10^{-5}$ and $1{,}800 \times 10^{-5}$ M glutamate are almost identical. This prediction indicates that whatever neutral form of cotransporter is available will be fully saturated by $50 \times 10^{-5}$ M glutamate.

Figure 2 compares computer predictions with experimental data from Fig. 7 (27 mM) of Wheeler and Graves (1983). In their experiment, synaptosomes were exposed to an external glutamate concentration of $1 \times 10^{-5}$ M and a sodium concentration of 27 mM at increasing potassium concentrations in order to depolarize the membrane. For the computer analysis, the following mechanisms were functional on the external side of the membrane: Both the positively charged and the neutral forms of the cotransporter were operating with $K_7$ decreasing as potassium increased. On the inside membrane, only the neutral form of the cotransporter was functional. The concentration-dependent leak of sodium glutamate was operating with a permeability coefficient of 0.12 $min^{-1}$. The sodium/potassium pump was programmed to keep synaptosomal sodium at 4.5 mM.

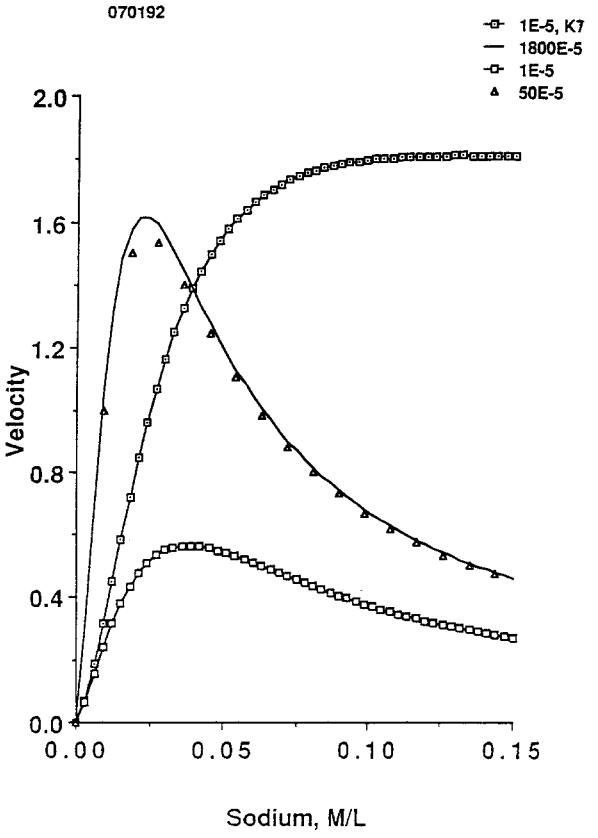



**Fig. 1.** A plot of initial velocity of glutamate uptake as a function of the sodium concentration.

The fit of prediction to experimental data was made by eye. The question, of course, may be asked whether this is the only solution. It is appropriate to note then what did not work and why. It may be said at the outset that the fit was very sensitive to the value of synaptosomal sodium chosen. Changes in sodium of 1 mM were catastrophic. Changes as small as 0.5 mM made significant shifts in the predicted curve. Figure 2 shows how the choice of 5 mM sodium and a leak coefficient of 0.10 $min^{-1}$ low-

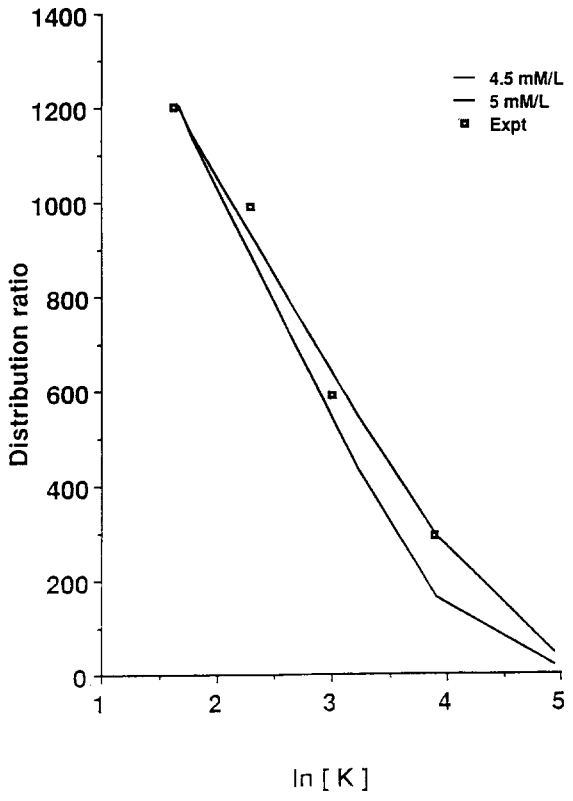



**Fig. 2.** A comparison of computer predictions with experimental data from Fig. 7 (27 mM) of Wheeler and Graves (1979, 1983).

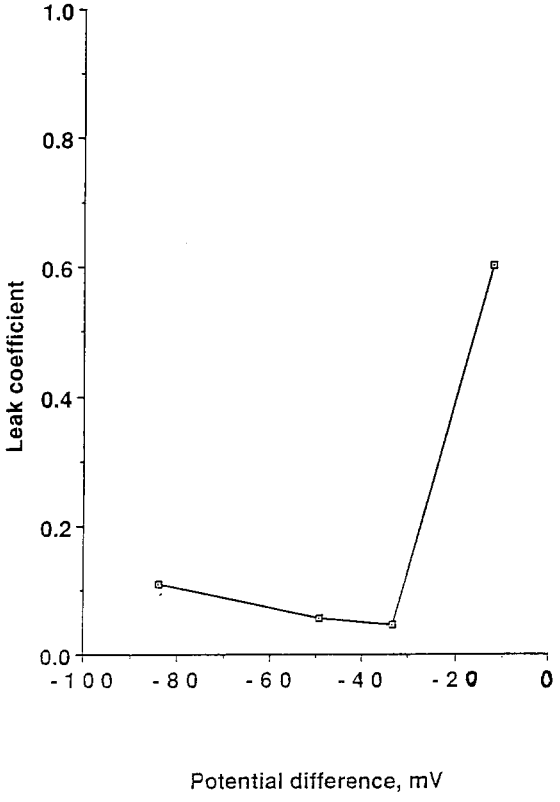



**Fig. 3.** A graph of predicted values of the permeability coefficient of sodium glutamate as a function of membrane potential.

ers the curve. The explanation for this sensitivity may be deduced from Fig. 1. The only way to accumulate glutamate inside the synaptosome is for the velocity of the cotransporter on the external surface to exceed the velocity of the cotransporter on the inner surface. This may be noted by the steepness of the curve which describes the velocity of the neutral form of the cotransporter on the inner surface of the membrane at $50 \times 10^{-5}$ or $1{,}800 \times 10^{-5}$ M. Small changes in synaptosomal sodium produced dramatic changes in the rate at which glutamate was being transported out of the synaptosome. Since the influx of glutamate and the leak are constant, the accumulation of glutamate would be affected by these small changes. Therefore, one may find only one value for synaptosomal sodium which will allow the accumulation of glutamate to reach the level determined experimentally.

Figure 1 also indicates what happens if the leak pathway is not functional and only the transporters on each side of the membrane are functioning at a medium sodium concentration of 27 mM and a synaptosomal sodium concentration of 4.5 mM. The velocity of entry is much greater than the velocity of exit. As a result, glutamate accumulates. The computer program predicted a curve of rising distribution ratio which did not level off. At 30 min, the distribution ratio predicted was 4,186, a value much larger than could be observed experimentally. Therefore, it was necessary to include a concentration-dependent outward movement.

What happens if the transporter is present only on the external side of the membrane and only the concentration-dependent leak and the pump are operating on the inner surface? The computer program was adjusted to eliminate the cotransporter mechanism on the inner surface of the membrane. The pump was adjusted to keep synaptosomal sodium at 4.5 mM. The permeability coefficient of the leak was adjusted so that the distribution ratio observed experimentally at each external potassium concentration would be reached. The object of the procedure was to determine whether there was any reasonable relationship between the permeability of the leak and the membrane potential at different potassium concentrations. Figure 3 graphs predicted values of the leak coefficient as a function of membrane potential. From −84.2 to −40 mV the permeability coefficient decreased as might be expected if the glutamate anion were moving down its electro-

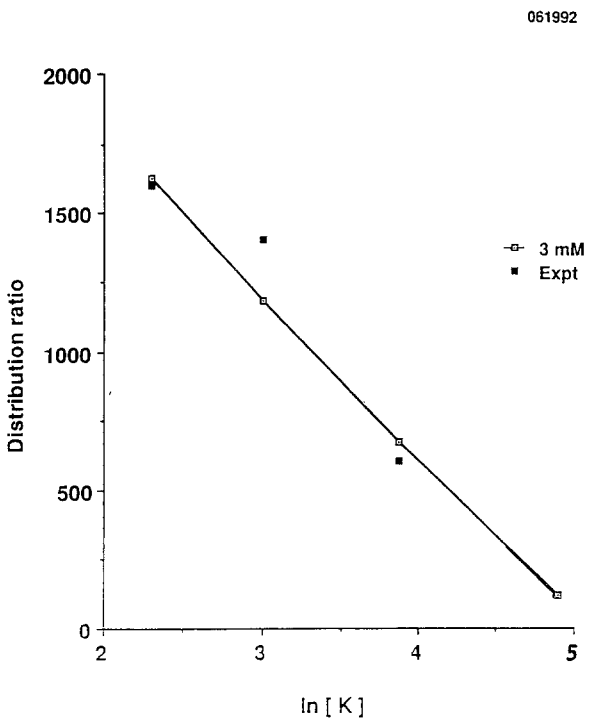



**Fig. 4.** A comparison of computer predictions with experimental data from Fig. 7 (127 mM) of Wheeler and Graves (1979, 1983).

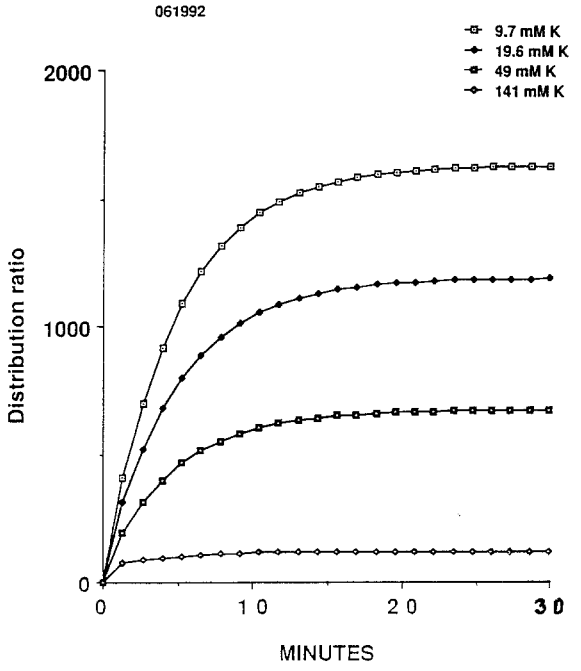



**Fig. 5.** A graph of the predicted distribution ratio as a function of time after synaptosomes were exposed to increasing external potassium concentration.

chemical gradient, carrying sodium along with it. However, this relationship no longer held when the membrane was depolarized further. Instead the permeability coefficient rose sharply. The explanation may be quite simple. Theoretically the leak coefficient might go to 0 at 0 mV, but the entry of glutamate into the synaptosomes cannot because at 0 mV the neutral form of the cotransporter is still operating. The end result, therefore, would be uncontrolled entry of glutamate. Obviously this does not occur. Therefore, it is unlikely that leak can operate alone to control the synaptosomal distribution ratio. It would appear that a symmetrical cotransporter on the inner surface is necessary.

The same procedure was used to fit the data of Fig. 7 (120.8 mM) from Wheeler and Graves (1983). This graph also was a plot of the distribution ratio *vs.* the logarithm of the potassium concentration. The fit was attempted only for the linear part of the curve, since the authors had indicated that the response to potassium at low concentrations was anomalous. The computer fit is shown in Fig. 4. The concentration of synaptosomal sodium was set at 3 mM and a leak coefficient of 0.225 $min^{-1}$.

Again the fit was very sensitive to the choice of synaptosomal sodium. Similarly, no reasonable correlation was found between the leak coefficient and the potential difference if the neutral form of the cotransporter was omitted. It would seem, therefore, that a symmetrical transporter on each side of the membrane is necessary under these experimental conditions as well.

Figure 5 is a graph of the predicted distribution ratio as a function of time after synaptosomes were exposed to increasing external potassium concentrations. The time course at 9.7 mM potassium may be compared with Fig. 5 of Wheeler and Graves (1983).

## Discussion

Wheeler and co-workers (1983) had shown that synaptosomes could accumulate glutamate to values as high as 1,800 times the medium. They also showed that free energy was available from the sodium concentration gradient and the membrane potential. An electrogenic cotransporter had to operate on the outer side of the membrane. It remained to be established what mechanisms may be operating on the inner surface of the membrane. The present model has shown that four mechanisms would be operating to fit the experimental data. The demands on these mechanisms were fixed. Experimental results required a linear relation between distribution ratio and the membrane potential. The only mechanism which was subject to this requirement was the influx of glutamate by the electrogenic form of the cotrans-

porter. The other mechanisms could be linear relationships or constants. Since the Fick equation is a linear relation between flux and concentration difference, the concentration-dependent flux of sodium glutamate met the linearity criterion. Figure 1 demonstrated that the neutral form of the glutamate cotransporter saturated at synaptosomal glutamate concentrations 50 times the medium glutamate concentration or higher. Therefore, the flux produced by this mechanism was constant for any given synaptosomal sodium concentration. Finally, this sodium concentration was kept constant by the operation of the sodium/potassium pump.

In this proposed scheme, the sodium/potassium pump carries out an additional function and that is to maintain electrical neutrality inside the synaptosome. At a distribution ratio of 1,800, for example, 18 mM of anion has entered the synaptosome. If the synaptosomal sodium concentration does not change, how is electrical neutrality maintained? One would have to conclude that part of the glutamate entry was matched by an increase in potassium ion and part by a loss of synaptosomal chloride. Changes in synaptosomal volume are also a consequence of glutamate accumulation unless all the gain of glutamate is matched by a loss of chloride or some other synaptosomal anion, The synaptosomes will gain osmotically active solute, water, and swell.

The sensitivity of glutamate accumulation to the concentration of sodium inside the synaptosomes emphasizes the importance of the sodium/potassium pump in the maintenance of synaptosomal sodium. Small changes in pump function could produce significant shifts in glutamate concentration and influence its function in neural tissue. In addition, the pump acts to limit the degree of depolarization of the membrane produced by the electrogenic form of the cotransporter. In turn, the depolarization produced by the electrogenic form of the cotransporter is self-limiting since its function is decreased as the membrane potential falls. Finally, the concentration-dependent leak serves to limit the extent of accumulation produced by an imbalance between the cotransporters on each side of the membrane. This generalized transport system seems to be a delicate balance designed for the internal regulation of this important neural metabolite.

One assumption remains moot in this analysis because of the lack of experimental evidence. The model has assumed that the concentration of the transporter is identical on each side of the membrane and does not change. The implication of this assumption is that the recycle time of the transporter is not rate-limiting. The other option is that transporters are fixed on their respective membrane sides and that the maximal flux is determined by the rate constant. Until experiments are carried out that measure initial influx as a function of internal glutamate concentration or vice versa, this question will not be answered.

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