

HIGH AFFINITY GLUTAMATE TRANSPORT IN SYNAPTOSOMAL PREPARATIONS — IS THERE A GLIAL CONTRIBUTION TO UPTAKE?

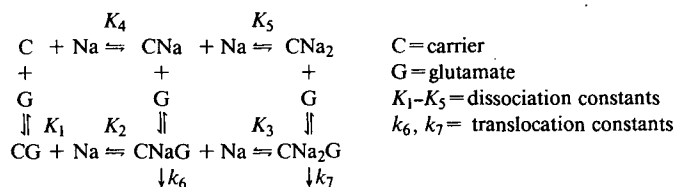
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Abstract

Previous studies have shown that kinetic data for glutamate uptake by cortical synaptosomal preparations give an excellent fit to a single-carrier model of transport. Since glial cells have also been shown to take up glutamate, the data have been re-examined and fitted to two-carrier models. No two-carrier model is found to fit the data as well as a single-carrier model. From these results and from comparisons of our data to published data for glial cells, it is concluded that glial cells probably do not make a measurable contribution to glutamate uptake by synaptosomal preparations.

In previous studies in this laboratory, we have examined the sodium dependence of synaptosomal transport of compounds which are thought to play a role in transmitter function (Wheeler and Hollingsworth, 1978, 1979; Wheeler, 1979a,b; Wheeler, 1980a,b,c; Wheeler, Wise and Callihan, 1980; Wheeler, 1981). We find that high affinity glutamate transport is essentially totally dependent on the presence of sodium in the incubation medium. Computer optimization techniques have been used to fit kinetic data (initial velocities) to several plausible models which would account for the remarkable sodium dependence, and the model giving minimal best fit has been identified (below).



The model gives a remarkably good fit to the data; the average error between experimental data points and model predictions is only about 1.5% (N = 12). As can be seen from the above model, translocation may take place as CNaG and as CNa₂G. Thus two sodium ions are translocated with each glutamate by one of the pathways, while only one sodium is translocated with each glutamate by the second pathway. At low [Na], the CNaG pathway predominates, while at higher [Na], the CNa₂G pathway predominates.

The following questions have been raised concerning these studies. Since uptake can take place by two pathways, could each pathway represent a separate transport mechanism? And since glial cells have been found to take up glutamate (Hertz, 1979), could glial uptake account for one of the mechanisms? If both synaptosomes and glia contribute to uptake, then measured uptake is the sum of the glial (1) and synaptosomal (2) contributions:

$$V_t = \frac{V_1[S]}{K_1 + [S]} + \frac{V_2[S]}{K_2 + [S]}$$

V_t = sum of initial velocities
 V_1, V_2 = apparent V_{max} 's
 K_1, K_2 = apparent K_m 's
 $[S]$ = substrate concentration

Although an excellent fit is obtained to a model composed of a single transport system, a better fit should be obtained to a model comprised of two carriers if indeed two carriers are present.

In order to determine whether the initial velocity data can be fitted to a model consisting of two separate transport systems, the single model was divided into two parts, and the data were fitted to each of these two-component models (Table 1). In dividing the large model, each of the two translocation steps is assumed to represent transport by a separate carrier mechanism.

Table 1
Possible Models

| Model | K_1 | K_2 | K_5 |
|-------|---------------------------------|-----------------------------------|---------------------------------|
| 1. | $C + Na \rightleftharpoons CNa$ | $+ Na \rightleftharpoons CNa_2$ | $C + Na \rightleftharpoons CNa$ |
| | + | + | + |
| | G | G | G |
| | K_3 | K_7 | K_6 |
| | CNa_2G | $CG + Na \rightleftharpoons CNaG$ | $CNaG$ |
| | ↓ k_4 | | ↓ k_8 |
| | Relative error = 1.4362 | | |
| 2. | $C + Na \rightleftharpoons CNa$ | $+ Na \rightleftharpoons CNa_2$ | C |
| | + | + | + |
| | G | G | G |
| | K_3 | K_5 | K_6 |
| | CNa_2G | $CG + Na \rightleftharpoons CNaG$ | $CNaG$ |
| | ↓ k_4 | | ↓ k_7 |
| | Relative error = 1.4656 | | |
| 3. | $C + Na \rightleftharpoons CNa$ | $+ Na \rightleftharpoons CNa_2$ | $C + Na \rightleftharpoons CNa$ |
| | + | + | + |
| | G | G | G |
| | K_3 | K_6 | K_6 |
| | CNa_2G | $CNaG$ | $CNaG$ |
| | ↓ k_4 | | ↓ k_7 |
| | Relative error = 1.4851 | | |

Table 1—continued

| Model | | |
|-------|---|---|
| 4. | $\begin{array}{c} \text{C} \\ + \\ \text{G} \\ \parallel K_1 \quad K_2 \\ \text{CG} + \text{Na} \rightleftharpoons \text{CNaG} + \text{Na} \rightleftharpoons \text{CNa}_2\text{G} \\ \downarrow k_4 \end{array}$ | $\begin{array}{c} \text{C} + \text{Na} \rightleftharpoons \text{CNa} \\ + \\ \text{G} \\ \parallel K_7 \quad \parallel K_6 \\ \text{CG} + \text{Na} \rightleftharpoons \text{CNaG} \\ \downarrow k_8 \end{array}$ |
| | Relative error = 5.4627 | |
| 5. | $\begin{array}{c} \text{C} \\ + \\ \text{G} \\ \parallel K_1 \quad K_2 \\ \text{CG} + \text{Na} \rightleftharpoons \text{CNaG} + \text{Na} \rightleftharpoons \text{CNa}_2\text{G} \\ \downarrow k_4 \end{array}$ | $\begin{array}{c} \text{C} + \text{Na} \rightleftharpoons \text{CNa} \\ + \\ \text{G} \\ \parallel K_6 \\ \text{CNaG} \\ \downarrow k_7 \end{array}$ |
| | Relative error = 5.5378 | |
| 6. | $\begin{array}{c} \text{C} \\ + \\ \text{G} \\ \parallel K_1 \quad K_2 \\ \text{CG} + \text{Na} \rightleftharpoons \text{CNaG} + \text{Na} \rightleftharpoons \text{CNa}_2\text{G} \\ \downarrow k_4 \end{array}$ | $\begin{array}{c} \text{C} \\ + \\ \text{G} \\ \parallel K_5 \quad K_6 \\ \text{CG} + \text{Na} \rightleftharpoons \text{CNaG} \\ \downarrow k_7 \end{array}$ |
| | Relative error = 30.4428 | |
| 7. | $\begin{array}{c} \text{C} + \text{Na} \rightleftharpoons \text{CNa} \\ + \\ \text{G} \\ \parallel K_1 \quad K_2 \quad \parallel \\ \text{CG} + \text{Na} \rightleftharpoons \text{CNaG} \\ \downarrow k_4 \end{array}$ | $\begin{array}{c} \text{C} + \text{Na} \rightleftharpoons \text{CNa} \\ + \\ \text{G} \\ \parallel K_8 \quad \parallel K_7 \\ \text{CNaG} + \text{Na} \rightleftharpoons \text{CNaG}_2 \\ \downarrow k_9 \end{array}$ |
| | Relative error = 1.4559 | |
| 8. | $\begin{array}{c} \text{C} + \text{Na} \rightleftharpoons \text{CNa} \\ + \\ \text{G} \\ \parallel K_4 \\ \text{CNaG} + \text{Na} \rightleftharpoons \text{CNa}_2\text{G} \\ \downarrow k_5 \end{array}$ | $\begin{array}{c} \text{C} \\ + \\ \text{G} \\ \parallel K_6 \quad K_7 \\ \text{CG} + \text{Na} \rightleftharpoons \text{CNaG} \\ \downarrow k_8 \end{array}$ |
| | Relative error = 1.4784 | |

Table 1—continued

| Model | | |
|-------|--|---|
| 9. | $\begin{array}{c} \text{C} + \text{Na} \rightleftharpoons \text{CNa} \\ + \\ \text{G} \\ \parallel K_4 \\ \text{CNaG} + \text{Na} \rightleftharpoons \text{CNa}_2\text{G} \\ \downarrow k_5 \end{array}$ | $\begin{array}{c} \text{C} + \text{Na} \rightleftharpoons \text{CNa} \\ + \\ \text{G} \\ \parallel K_3 \\ \text{CNaG} + \text{Na} \rightleftharpoons \text{CNa}_2\text{G} \\ \downarrow k_8 \end{array}$ |
| | Relative error = 2.0269 | |
| 10. | $\begin{array}{c} \text{C} + \text{Na} \rightleftharpoons \text{CNa} \\ + \\ \text{G} \\ \parallel K_2 \quad K_3 \\ \text{CNaG} + \text{Na} \rightleftharpoons \text{CNa}_2\text{G} \\ \downarrow k_4 \end{array}$ | $\begin{array}{c} \text{C} \\ + \\ \text{G} \\ \parallel K_5 \quad K_6 \\ \text{CG} + \text{Na} \rightleftharpoons \text{CNaG} \\ \downarrow k_7 \end{array}$ |
| | Relative error = 28.7291 | |
| 11. | $\begin{array}{c} \text{C} + \text{Na} \rightleftharpoons \text{CNa} \\ + \\ \text{G} \\ \parallel K_2 \quad K_3 \\ \text{CNaG} + \text{Na} \rightleftharpoons \text{CNa}_2\text{G} \\ \downarrow k_4 \end{array}$ | $\begin{array}{c} \text{C} + \text{Na} \rightleftharpoons \text{CNa} \\ + \\ \text{G} \\ \parallel K_6 \\ \text{CNaG} \\ \downarrow k_7 \end{array}$ |
| | Relative error = 6.2750 | |
| 12. | $\begin{array}{c} \text{C} + \text{Na} \rightleftharpoons \text{CNa} \\ + \\ \text{G} \\ \parallel K_2 \quad K_3 \\ \text{CNaG} + \text{Na} \rightleftharpoons \text{CNa}_2\text{G} \\ \downarrow k_4 \end{array}$ | $\begin{array}{c} \text{C} + \text{Na} \rightleftharpoons \text{CNa} \\ + \\ \text{G} \\ \parallel K_5 \quad K_6 \quad \parallel \\ \text{CG} + \text{Na} \rightleftharpoons \text{CNaG} \\ \downarrow k_8 \end{array}$ |
| | Relative error = 5.6661 | |

Even though some of the two carrier models contain more constants, none of them fits the data as well as the single carrier model. The relative error ranges from 1.44 for model 1 to 30.44 for model 6. At higher [Na]'s, velocity of uptake falls off as the [Na] is increased (Fig. 1). Therefore, any representative model must reflect this inhibitory effect of high [Na]. When the best fit constants for each of the models are entered into the rate equation and predicted velocities are calculated, none of the two-carrier models predicts this sodium effect. However, the single

carrier model accurately predicts this behavior (Fig. 1). This is compelling evidence that the single carrier model more accurately fits the data than any of the two-carrier models in Table 1.

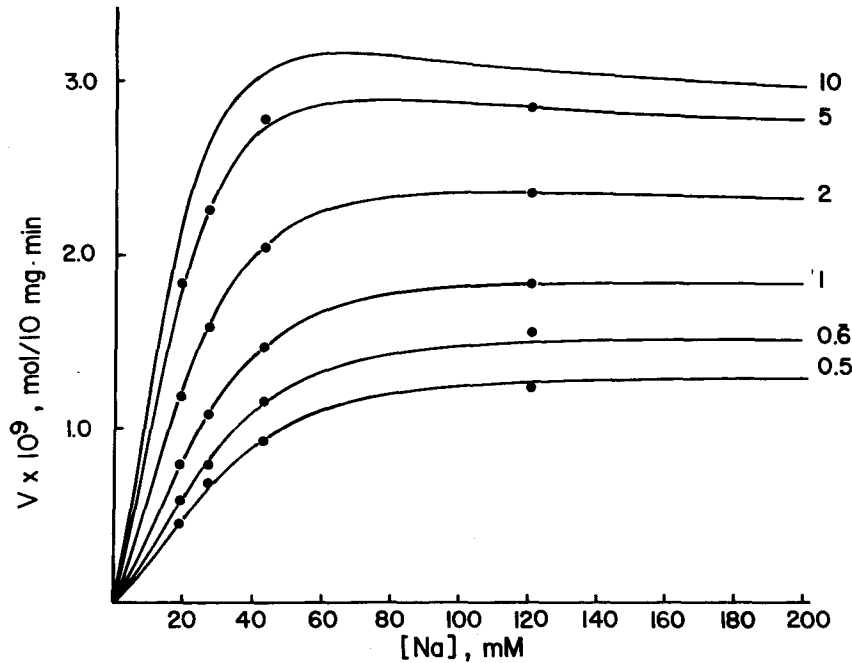


Fig. 1. Velocity of uptake as a function of sodium concentration for each of the glutamate concentrations used. Lines show the computed fit to the minimal best fit model, while symbols show the experimental data points. $[G] \times 10^5$ M is given to the right of each curve.

There are other indications that a single carrier fits the data better than two. If two carriers contribute to uptake, then

$$V_i = \frac{V_1[S]}{K_1 + [S]} + \frac{V_2[S]}{K_2 + [S]}$$

and,

$$\frac{1}{V_i} = \frac{K_1 K_2 + K_2[S] + K_1[S] + [S]^2}{K_2 V_1[S] + V_1[S]^2 + K_1 V_2[S] + V_2[S]^2}$$

Thus Lineweaver-Burk plots should not be linear. However, the data give excellent linear fits, with correlation coefficients of 0.9949 for 120.8mM $[Na]$ to 0.9993 for 19mM $[Na]$ (not shown). There is, however, one case in which two carriers would give linear fits to reciprocal plots — if the apparent K_i were the same for both carriers. If $K_1 = K_2 = K$, then

$$V_i = \frac{V_1[S]}{K + [S]} + \frac{V_2[S]}{K + [S]} = \frac{V_1[S] + V_2[S]}{K + [S]}$$

$$\frac{1}{V_i} = \frac{K}{(V_1 + V_2)} \left(\frac{1}{[S]} \right) + \frac{1}{V_1 + V_2}$$

Thus a plot of $1/V$ versus $1/[S]$ would yield a straight line. At normal $[Na]$'s, we calculate K_i to be about $6 \mu M$. K_i 's for glial cells are much higher. Bulk prepared astrocytes, which are prepared by gradient centrifugation, and should thus be most comparable to a synaptosomal preparation, have been reported to have K_i 's from 10 – $12 \mu M$ (Henn, 1976; Henn *et al.*, 1974; Weiler *et al.*, 1979). Values from 10 – $220 \mu M$ have been reported for astrocytes in primary culture (Schousboe *et al.*, 1977; Balcar and Hauser, 1978; Hertz *et al.*, 1978). Such large differences in K_i 's should be reflected in non-linear reciprocal plots.

Other comparisons of our data to data in the literature for astrocytes also argue against a substantial contribution of glial cells to uptake by synaptosomal preparations. At a $[G]$ of $5 \mu M$, we obtain an uptake of about $0.88 \mu mol/min$ per gram of synaptosomes, while Schousboe and Divac (1979) report a value of about $0.01 \mu mol/min$ per gram for astrocytes in primary culture (assuming 100 mg protein/g wt weight). Thus the astrocyte contribution would not be detectable. We find V_a at normal $[Na]$ to be about $2.16 \mu mol/min$ per gram of synaptosomes, while values for V_a of only 0.06 have been reported for bulk-prepared astrocytes (Weiler *et al.*, 1979). However, it should be noted that V_a 's as high as $5.9 \mu mol/min$ per gram with a K_i of about $50 \mu M$ have been reported for astrocytes in primary culture (Hertz *et al.*, 1978). Thus there is a hundred fold difference between reported values for bulk-prepared astrocytes and astrocytes in primary culture. As pointed out above, there are also large differences in reported K_i 's for bulk-prepared astrocytes (10 – $12 \mu M$) and astrocytes in primary culture (10 – $220 \mu M$). Thus kinetic constants determined for cultured astrocytes may not be representative of astrocytes under *in vivo* conditions. The kinetic constants reported for astrocytes in primary culture differ vastly from study to study. For example, Balcar and Hauser (1978) report a K_i of 10 – $20 \mu M$ and a V_a of 0.4 – $0.6 \mu mol/min/g$; Hertz *et al.* (1978) report a K_i of $50 \mu M$ and V_a of $5.9 \mu mol/min/g$; while Schousboe *et al.* (1977) report a K_i of $220 \mu M$ and V_a of $0.8 \mu mol/min/g$. At a $[G]$ of $5 \mu M$, Schousboe and Divac (1979) report an uptake of between 0.078 and 0.13 nmol/min per mg cell protein, while Hertz, Schousboe, Boechler, Mukerji, and Federoff (1978) report a value of about 5 nmol/min per mg cell protein. Thus the same author reports values which differ by 50 fold. The large differences between values reported for bulk-prepared astrocytes and astrocytes in primary culture, and the widely divergent values for astrocytes in primary culture, would seem to indicate that one should be cautious about interpreting results from such cultured preparations. One should also be careful in comparing results from vesicle preparations such as synaptosomes with results from cellular preparations, such as astrocytes in primary culture or bulk-prepared astrocytes. Comparison of apparent K_i 's would seem to be quite legitimate, since K_i does not depend on tissue mass. However, caution should be observed in com-

parisons of apparent maximal velocity (V_a), since V_a depends on the normalization factor used.

In conclusion, the data give best fit to a one-carrier model. If two carriers are involved, then their K_t 's must be very similar. However, K_t 's from astrocyte preparations are considerably higher than that which we obtain for synaptosomes, which would argue against a measurable astrocytic contribution to uptake in synaptosomal preparations.

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