

A MODEL OF GABA TRANSPORT BY RAT CORTICAL SYNAPTOSOMES: FURTHER STUDIES

D. D. WHEELER*

*Department of Physiology, Medical University of South Carolina,
171 Ashley Avenue, Charleston, South Carolina 29425, U.S.A.*

(Received March 11, 1981)

Abstract

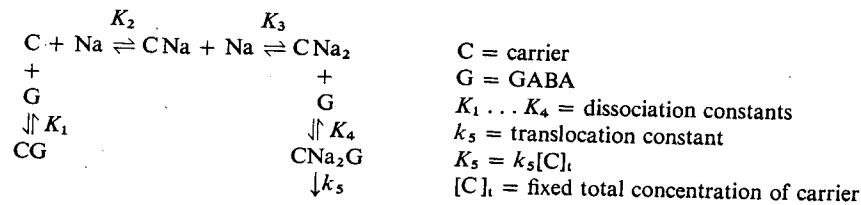
Further kinetic studies have been made of the sodium dependence of GABA transport by rat cortical synaptosomes. The method of blank determination has been improved, studies of uptake in zero sodium medium have been made over a wide range of GABA concentrations, and the number of kinetic experiments has been doubled. The composite data from all kinetic studies have been fitted by computer optimization techniques to several plausible models, and the minimal best fit model has been identified. This model differs in some aspects from the models which have been described in previous studies. The kinetic constants which quantitate the model have been estimated and utilized along with the associated rate equation for the model to define and compute certain parameters which are useful in describing and comparing transport mechanisms. Some of these parameters are very similar to values estimated from previous studies, while others are considerably different. The most important difference in the model presented and previous models is in mechanism.

1. Introduction

In previous studies in this laboratory, we have examined the remarkable sodium dependence of high affinity GABA transport by cortical synaptosomal preparations (Wheeler and Hollingsworth, 1979; Wheeler, 1980*a,b*). Computer optimization techniques were used to fit kinetic data (initial velocities) to several plausible models which would account for the extreme sodium dependence, and the minimal best fit model was identified. Since uptake at $[Na] = 0$ was found to be so small compared to that when the incubation medium contained 120.8 mM sodium (an average of only 1.79%), the initial modelling studies did not allow

* Supported by Grant No. NS-12292 from the National Institutes of Health.

translocation in the carrier-GABA form (Wheeler and Hollingsworth, 1979). Under these conditions, the following minimal best fit model emerged:



$$v = \frac{K_5[\text{Na}]^2[\text{G}]/(K_2K_3K_4)}{1 + \frac{[\text{G}]}{K_1} + \frac{[\text{Na}]}{K_2} + \frac{[\text{Na}]^2}{K_2K_3} + \frac{[\text{Na}]^2[\text{G}]}{K_2K_3K_4}}$$

It was later discovered that even though uptake is close to zero when $[\text{Na}] = 0$, a better fit to the data was obtained when translocation as CG was allowed (Wheeler, 1980b). Thus it appeared that there might be a measurable uptake in the absence of sodium.

2. Methods

Preparation of synaptosomes

Adult, male Long-Evans rats were used in all experiments. Following decapitation with a guillotine, the brain was removed and placed on a filter paper moistened with Krebs-Henseleit solution (Lockwood, 1961) which was kept cold by placing on a petri dish filled with ice. The cortex was rapidly dissected free, weighed, and placed in 20 volumes of ice cold 0.32 M sucrose in a 15-ml Potter-Elvehjem mortar fitted with a Teflon pestle. The clearance between mortar and pestle was 0.004–0.006 in. The homogenizer was placed in an ice water bath and the tissue homogenized for one minute, utilizing six 10-second strokes with a speed of 840 rpm. The homogenate was centrifuged for 10 minutes at $1085 \times g$ at a temperature of 2°C to remove nuclei and cellular debris. The supernate was removed and centrifuged at $27,000 \times g$ for 15 minutes to sediment the synaptosomes. The supernate from this procedure was decanted and discarded, and the synaptosomes were resuspended in 20 volumes of ice cold 0.32 M sucrose.

Measurement of uptake

The uptake of 14-C labelled γ -aminobutyric acid was measured by incubation of synaptosomes for one minute in a modified Krebs-Henseleit solution at 30°C. It has been shown in studies utilizing similar preparations that more than 95–98% of the GABA taken up is recoverable as GABA after 10 minutes of incubation (Martin and Smith, 1972; Ryan and Roskoski, 1977). Thus the amount of metabolism during one minute of incubation would be negligible. 14-C labelled GABA (U.L., specific activity of 192 mCi/m-mol) was obtained from the New England Nuclear Corporation, Boston, MA, and was at least 98% pure. Non-labelled

GABA was obtained from Sigma Chemical Company, St Louis, MO. All incubations were carried out in a Dubnoff metabolic shaker. The modified Krebs-Henseleit used consisted of 127.2 mM NaCl, 5.0 mM KCl, 2.7 mM CaCl₂, 1.3 mM MgSO₄, 25 mM Tris buffer, and 11.1 mM glucose, at a pH of 7.4. The concentration of GABA in the final incubation medium was 5.0, 2.0, 1.0, 0.6667, or 0.5×10^{-6} M. For each sodium concentration used, a solution containing the highest amino acid concentration was prepared with a specific activity of 1×10^7 $\mu\text{Ci/mol}$ and serially diluted; thus a constant specific activity was maintained at all amino acid concentrations. Concentration of sodium in the incubation medium was varied by replacement of NaCl with equiosmolar Tris buffer; 0, 19.0, 27.0, 43.0, and 120.8 mM sodium solutions were used with each of the above GABA concentrations. Incubation was begun by pipetting 0.2 ml of the synaptosomal suspension (equivalent to 10 mg of cortical tissue) into a scintillation vial containing 3.8 ml of incubation medium.

Following incubation, synaptosomes were separated from incubation medium by rapid filtration, utilizing a Millipore 30-place manifold and Millipore filters (HAWP02500). The synaptosomes were then washed with a 5-ml aliquot of Krebs-Henseleit ($[\text{Na}] = 0$) at 30°C. Previous studies indicate that such a wash procedure results in little loss of label from within the synaptosomes (Wheeler, 1978). Following completion of all incubations, each filter was removed, the bottom side blotted on filter paper, placed in a scintillation vial, and 1 ml distilled water added. After sitting for one hour, scintillation fluid was added; 24 hours later, the vials were counted for radioactivity in a Packard Model 3320 liquid scintillation spectrometer until the counting error was less than 1% at the 95% confidence level. Disintegrations per minute were calculated from counts per minute by use of an external standard to determine counting efficiency.

Initial velocities for each of the amino acid concentrations with each of the several sodium concentrations were measured with synaptosomes from a single preparation of tissue from one animal. The measurements were then repeated on 11 additional animals.

Blanks

In order to correct for filter retention of label when incubation medium is poured through them, blanks were run with each experimental solution. A 3.8-ml aliquot of incubation medium was added to a well of the filtration manifold, followed by the usual wash procedure. Filters were then treated as described above. Twenty-three repetitions were done with each GABA concentration. The amount of GABA trapped by the filters was computed and plotted as a function of GABA concentration. The data were then fitted to a straight line by linear regression; predicted values from this fit were determined for each GABA concentration and used for blank values. Initial velocity measurements were corrected by subtracting the blank value.

Computer optimization

In order to fit the data to the velocity equations for the various models, a parameter optimization approach was used to minimize the following function:

$$J = \sum_{i=1}^n \left(\frac{V_{\text{model}} - V_{\text{data}}}{V_{\text{data}}} \right)^2$$

J , the objective function, is the sum of the squared fractional errors between the velocities of uptake predicted by the models and those observed experimentally. The parameter optimization algorithm used in this research was based on the Flexible Polyhedron approach of Nelder and Mead (1965). The algorithm was coded in BASIC and run on a Challenger II microcomputer (Ohio Scientific Instruments) fitted with 48K bytes of memory and a dual drive floppy disk.

To use the BASIC program, the appropriate model equation and velocity data were first added to the optimization portion of the program. Initial guesses for all kinetic constants were then entered, and program operation was begun. After the algorithm returned the optimal parameters, the program was restarted several times to insure that the minimum possible error had been achieved. Finally, the program printed the various data velocities paired with the corresponding values predicted by the equations.

3. Results

In an attempt to determine whether measured uptake in zero [Na] medium truly represents uptake and not simply experimental error, three approaches have been used. First, since the difference between synaptosomal uptake from zero sodium medium and that trapped by the filters (which are used to separate synaptosomes from incubation medium) when incubation medium only is passed through them is so small, it is necessary to minimize fluctuations in the filter blanks. Therefore, the number of blank determinations has been increased from 5 to 23 for each GABA concentration used. Over the range of GABA concentration used (0.5×10^{-6} M to 5×10^{-6} M), the amount of GABA retained by the filter was found to be linearly related to the GABA concentration. Therefore, the data were fitted to a straight line by linear regression, and predicted values from this fit were then determined and used for blank values. In previous studies, the measured blank value for each GABA concentration was subtracted directly from the appropriate total uptake to obtain net synaptosomal uptake; no curve fitting of the blanks was done. Second, the number of kinetic experiments has been doubled so that data are now available from 12 animals. The improvement in blank accuracy and the doubling of the number of kinetic experiments resulted in a decrease in the average ratio of uptake in zero [Na] medium to that in 120.8 mM [Na] from 1.79% to only 0.84%. Thus it appears that as experimental error is reduced, measured uptake in zero sodium medium more closely approaches zero. If, however, this minimal uptake in zero sodium medium were real, it seemed

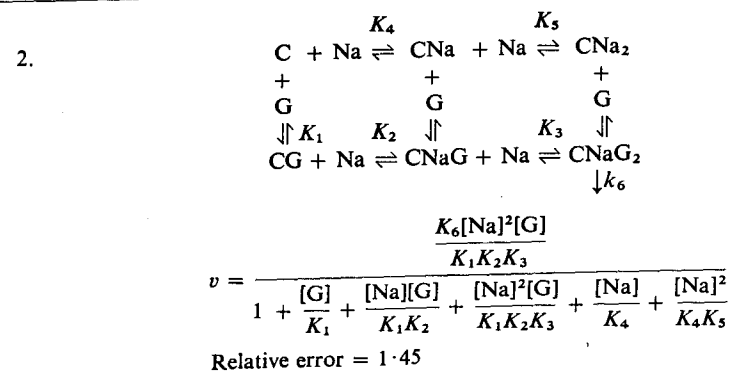
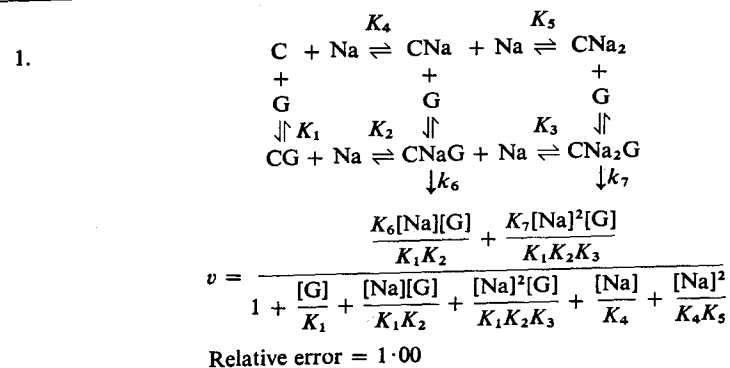
plausible that this might represent diffusive uptake, in which case uptake should increase linearly with GABA concentration. Therefore, in the third approach, uptake was measured in zero sodium medium containing GABA at a concentration of 40, 80, or 200×10^{-6} M and 14-C-labelled GABA at a specific activity of 2.5×10^6 μ Ci/mole. In 15 experiments at each GABA concentration, no measurable uptake was found. Thus any uptake in zero sodium medium is miniscule and within the error of the experimental technique.

Minimal best fit model

Having established that uptake in zero sodium medium is unimportant and having reduced the experimental error by increasing the number of experiments and improving the blank procedure, the data for [Na]'s of 19, 27, 43, and 120.8 mM were fitted to models 1–4 shown in Table 1. The data were first fitted to model 1, which allows translocation as CNaG and CNa₂G, but not as CG. An excellent

Table 1
Models

Model



GABA concentration which gives a velocity equal to $V_{\infty}/2$

If only [Na] is infinite, the rate equation reduces to

$$(2) \quad v = \frac{K_7[G]}{\frac{K_1K_2K_3}{K_4K_5} + [G]} = J_m$$

If $K_1K_2K_3/(K_4K_5) = [G]$, then

$$(3) \quad v = \frac{K_7[G]}{2[G]} = \frac{K_7}{2}$$

and the velocity of uptake is equal to half that at infinite [G] and [Na]. In the present study, a [G] of 3.02×10^{-6} M satisfies these conditions for model 1, while previous studies gave values of 1.91, 1.88, and 2.97×10^{-6} M for models 2, 4, and 5, respectively.

V_a , apparent maximal velocity

Velocity of uptake at infinite [G] is given by the following equation:

$$(4) \quad V_a = \frac{K_3K_6[Na] + K_7[Na]^2}{K_2K_3 + K_3[Na] + [Na]^2}$$

Fig. 1 shows a plot of V_a as a function of [Na] for model 1. At [Na] = 0, $V_a = 0$; as [Na] is increased from 0, V_a increases along an S-shaped curve, reaches a peak, and then declines with further increases in [Na] to approach K_7 as a limiting value. Over the range of [Na]'s used in these experiments, V_a for model 1 is quite

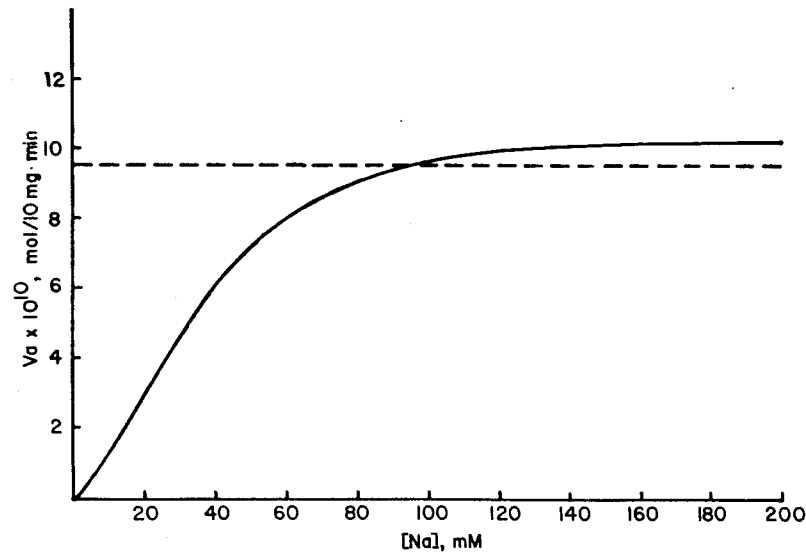


Fig. 1. The relationship between apparent maximal velocity of uptake (V_a) and sodium concentration. K_7 is given by the broken line.

close to previous estimates for models 2, 4, and 5 (Table 3). The [Na] at which V_a is a maximum can be obtained by setting the first derivative of the above equation equal to zero, and solving the resultant quadratic equation for [Na]:

$$(5) \quad K_3K_7[Na]^2 - K_3K_6[Na] + 2K_2K_3K_7[Na] + K_2K_3^2K_6 = 0.$$

The real, positive solution to this equation is 212.3 mM. If we insert this [Na] into equation 4, we obtain a V_a of 10.17×10^{-10} mol/10 mg min. Thus the true maximal velocity occurs not at infinite [Na], but at a [Na] of 212.3 mM. Further increases in [Na] above this value result in a reduction in V_a , so that V_a approaches $K_7 = 9.60 \times 10^{-10}$ mol/10 mg min at infinite [Na]. At [Na] = 140 mM, V_a is 10.03×10^{-10} mol/10 mg min. Thus at physiological [Na], the carrier is functioning at close to its maximal capacity with regard to [Na].

Table 3
Computed parameters which are Na-dependent

[Na], mM	V_a^*				K_t^*			
	M-1†	M-2	M-4	M-5	M-1	M-2	M-4	M-5
0	0	0	0	0.15	7.13	2.52	2.01	4.15
19	2.83	2.31	2.24	2.35	6.59	5.02	4.76	5.45
27	4.19	3.82	3.80	3.93	6.18	5.36	5.28	5.61
43	6.46	6.29	6.48	6.68	5.36	5.34	5.48	5.50
120.8	9.89	10.18	10.81	11.24	3.61	3.79	4.00	4.28
140	10.03	10.84	11.09	11.51	3.48	3.63	3.75	4.12
∞	9.60	11.41	11.98	12.47	3.02	1.91	1.88	2.97

* Units of V_a are 10^{-10} mol/10 mg min; units of K_t are micromolar.

† M-1 signifies model 1, etc.

Apparent Michaelis-Menten constant

K_t , the GABA concentration giving a velocity of uptake equal to $V_a/2$, is defined by the model as follows:

$$(6) \quad K_t = \frac{K_1K_2K_3K_4K_5 + K_1K_2K_3K_5[Na] + K_1K_2K_3[Na]^2}{K_2K_3K_4K_5 + K_3K_4K_5[Na] + K_4K_5[Na]^2}$$

Thus K_t is also dependent on the [Na]. At [Na] = 0, the equation reduces to $K_t = 7.13 \times 10^{-6}$ M. As [Na] is increased, K_t declines along an S-shaped curve, and approaches $K_1K_2K_3/(K_4K_5) = 3.02 \times 10^{-6}$ M (Fig. 2). Thus the apparent carrier affinity for GABA increases as the [Na] is increased, since apparent affinity is reciprocally related to K_t . $K_t(\text{Na} = 0)/K_t(\text{Na} = 140 \text{ mM}) = 2.05$ and $K_t(\text{Na} = \infty)/K_t(\text{Na} = 140 \text{ mM}) = 0.868$; thus the apparent affinity at physiological [Na] is twice that at zero [Na] and 86.8% of that at infinite [Na]. Comparison of K_t 's for model 1 to previous estimates for models 2, 4, and 5 (Table 3) reveals similar values except at very low and very high [Na]'s.

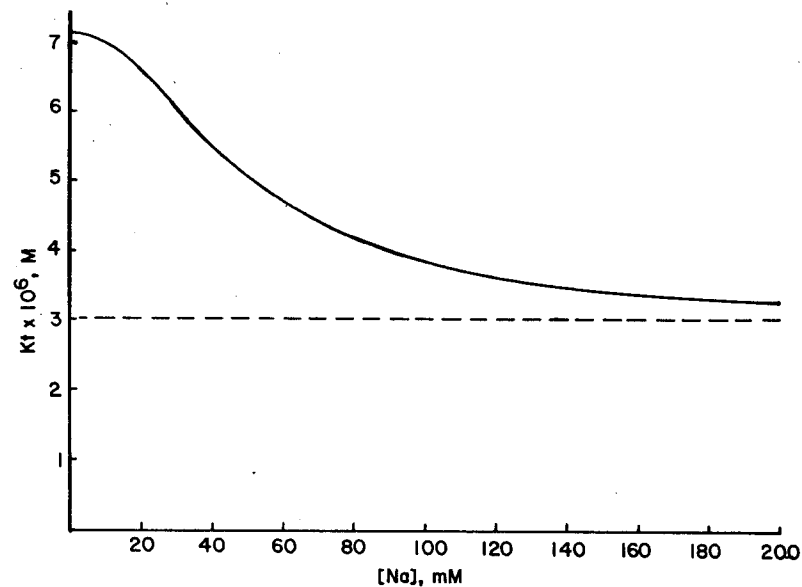


Fig. 2. K_t , the GABA concentration giving a velocity of uptake equal to $V_a/2$, as a function of sodium concentration. Broken line shows the limit at $[Na] = \infty$ which is given by $K_1K_2K_3/(K_4K_5)$.

J_m , rate of uptake at infinite $[Na]$ and a given $[G]$

J_m is defined by equation (2); as $[G]$ is increased, J_m increases along a hyperbolic curve and approaches K_7 as a limit at infinite $[G]$ (Fig. 3). J_m 's for model 1 are somewhat lower than previous estimates for models 2, 4, and 5 (Table 4). At a $[G]$ of 1×10^{-6} M and large $[Na]$, the carrier functions at 25% of its maximal rate at infinite $[G]$, since $J_m/K_7 = 2.39/9.60 = 0.25$.

κ_{Na} , the $[Na]$ which gives a velocity of uptake equal to $J_m/2$

K_t is a measure of the carrier sensitivity for GABA; analogously, κ_{Na} is a measure of the carrier sensitivity to sodium. Although κ_{Na} can be found by solving the rate equation for $[Na]$ when $v = J_m/2$, κ_{Na} can be found more easily from Fig. 4 by dropping a perpendicular line to the x-axis from the intersection of $J_m/2$ with the plot for a particular $[G]$. κ_{Na} declines as $[G]$ increases, from 52.6 mM at a $[G]$ of 0.5×10^{-6} M to 41.3 mM at a $[G]$ of 5×10^{-6} M. Thus at 140 mM $[Na]$, the carrier functions at a rate much higher than $J_m/2$. Estimates of κ_{Na} for model 1 are considerably less than previous estimates for models 2, 4, and 5, particularly at lower values of $[G]$ (Table 4).

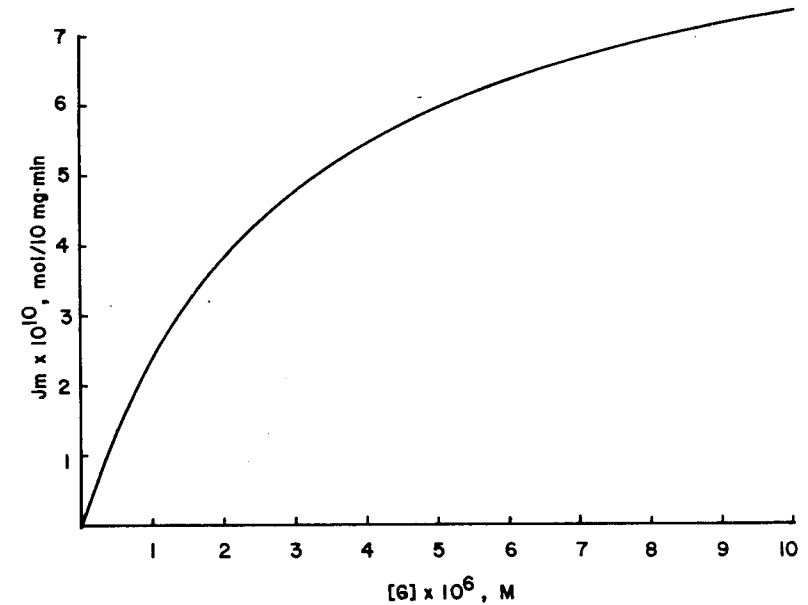


Fig. 3. The relationship between J_m , uptake at infinite $[Na]$, and GABA concentration.

Table 4
Computed parameters which are GABA-dependent

$[G] \times 10^6$ M	J_m^*				κ_{Na}^*			
	M-1	M-2	M-4	M-5	M-1	M-2	M-4	M-5
0.5	1.36	2.39	2.52	1.81	52.6	120.7	131.1	74.9
0.6	1.74	2.98	3.14	2.30	51.7	114.5	124.0	73.1
1.0	2.39	3.93	4.16	3.12	50.2	104.5	112.5	70.1
2.0	3.83	5.82	6.18	5.01	46.8	85.3	90.6	63.5
5.0	5.99	8.27	8.71	7.81	41.3	62.8	65.4	54.2

* Units of J_m are 10^{-10} mol/10 mg min; units of κ_{Na} are millimolar.

Velocity as a function of sodium concentration

We can use the rate equation from model 1, along with the best fit constants (Table 2), to compute velocity as a function of either $[Na]$ or $[G]$. Fig. 4 shows a plot of predicted velocity as a function of $[Na]$. Note how well the predicted curves fit the experimental data. The average percentage error is only 1.42%. Over the range of $[Na]$'s used, velocity increases with $[Na]$ along a sigmoidal curve for all $[G]$'s; at 200 mM $[Na]$, the curves have begun to plateau. At infinite $[Na]$,

velocity of uptake equals J_m . If one compares J_m (Table 4) with the predicted velocity shown in Fig. 4, one finds that for $[G]$'s of 2 and 5×10^{-6} M, J_m is lower than the predicted velocity for 200 mM $[Na]$. Thus at some $[Na]$, velocity passes through a maximum value and then declines to approach J_m . However, these peaks occur at $[Na]$'s far in excess of physiological $[Na]$. At 140 mM $[Na]$, velocity is close to that at infinite $[Na]$ for each $[G]$.

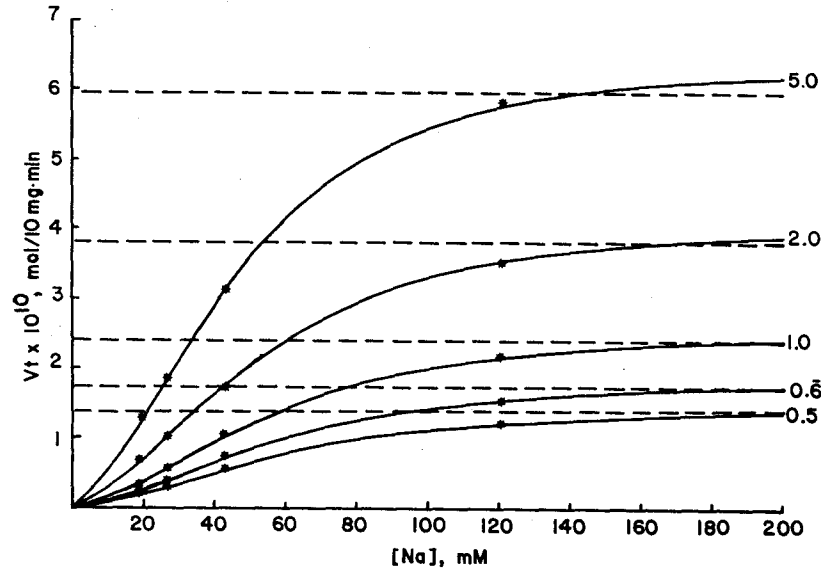


Fig. 4. Velocity of uptake as a function of sodium concentration for each GABA concentration. Number to the right of each curve gives the GABA concentration $\times 10^6$ M. Broken lines give the limits of the curves at infinite $[Na]$. Symbols show experimental data points.

Contribution of each pathway to total uptake

Translocation can take place in either of two forms: $CNaG$ or CNa_2G . Since $K_6 = k_6[C]_t$ and $K_7 = k_7[C]_t$, then $k_6/k_7 = K_6/K_7 = 4162 \cdot 2/96 \cdot 01 = 43 \cdot 4$. Thus the rate constant for translocation of the $CNaG$ species is much greater than that for the CNa_2G species. Does this mean that uptake is predominately through the $CNaG$ form? We can evaluate the contribution of each pathway by dividing the rate equation into its two components:

$$v_t = V_1 + V_2$$

$$(7) \quad v_t = \frac{K_6[Na][G]}{K_1K_2} + \frac{K_7[Na]^2[G]}{K_1K_2K_3},$$

D = denominator of the rate equation. Fig. 5 shows plots of computed velocity through each pathway as a function of $[Na]$ for each $[G]$ used. For uptake as

CNa_2G (V_2), a family of sigmoidal curves results. However, for uptake as $CNaG$ (V_1), velocity initially rises with increasing $[Na]$, but reaches a peak and then declines with further increases in $[Na]$. Thus declining uptake through this pathway is responsible for the inhibitory effect of high $[Na]$ which is demonstrated in Fig. 4.

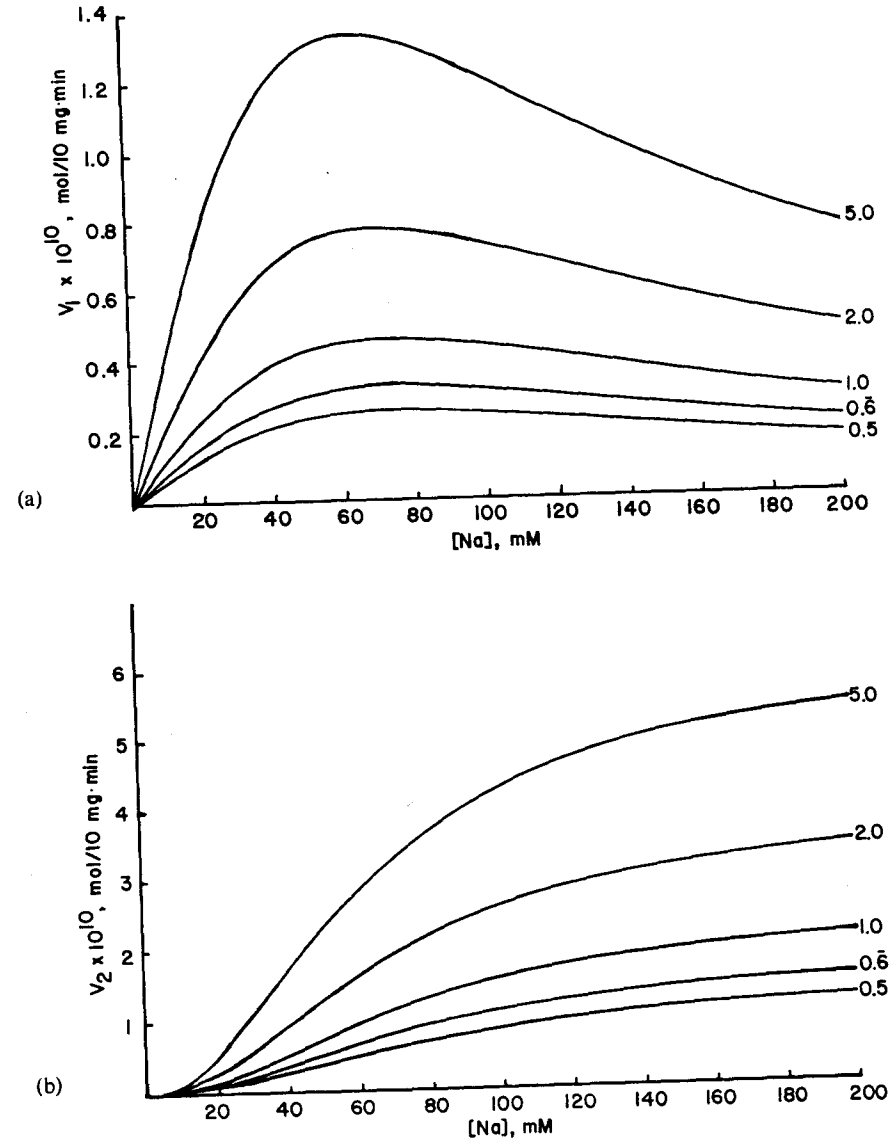


Fig. 5. Predicted velocities by each of the two pathways as a function of sodium concentration for each GABA concentration used. Number to the right of each curve gives the GABA concentration $\times 10^6$ M. (a) Contribution by pathway 1. (b) Contribution by pathway 2.

The relative importance of the two pathways to uptake can be appreciated by dividing the equation for V_2 by that for V_1 :

$$(8) \quad \frac{V_2}{V_1} = \frac{K_7[\text{Na}]}{K_3K_6}$$

Thus the ratio of the rates of uptake is independent of the $[\text{G}]$, but varies with the $[\text{Na}]$ (Figure 6). At 19 mM $[\text{Na}]$, $V_2/V_1 = 0.739$, while at 140 mM $[\text{Na}]$, $V_2/V_1 = 5.45$. Expressed as a percentage of total uptake, uptake as CNaG and as CNa_2G , respectively are: 57.50, 42.50; 48.78, 51.22; 37.42; 62.58; 17.55, 82.45, and 15.52, 84.48: at $[\text{Na}]$'s of 19, 27, 43, 120.8, and 140 mM, respectively. Thus at low $[\text{Na}]$'s a majority of total uptake takes place as CNaG , while at physiological $[\text{Na}]$, uptake as CNa_2G overwhelmingly predominates.

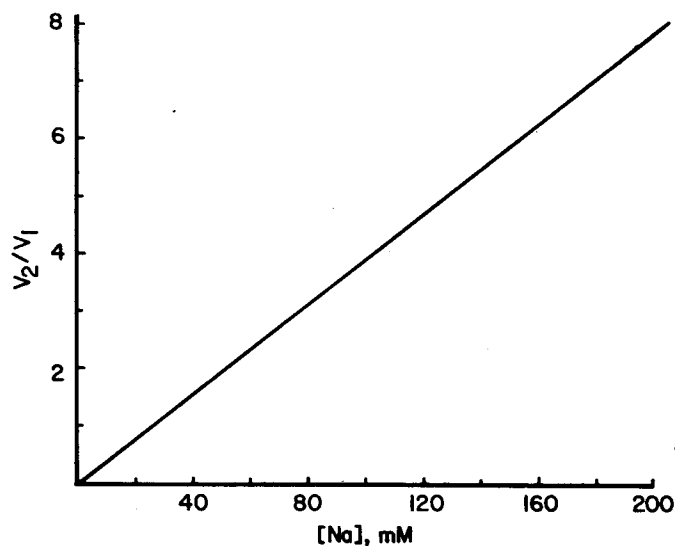


Fig. 6. Ratio of the uptake by the CNa_2G pathway to that by the CNaG pathway as a function of sodium concentration.

4. Discussion

It can now be reasonably concluded that uptake in zero sodium medium is either zero or so low as to be within the experimental error of the studies. Therefore, it is unnecessary to include translocation as CG in the model. Under these conditions, model 1 gives a much better fit to the data than either of the other models tested. The fit of the data to this model is excellent; the average percentage error between experimental data and model predictions is only 1.42%. The validity of the model is further enhanced by the fact that the number of experiments has been doubled and by the improvement in the blank procedure.

In general, the calculated parameters for model 1 are not dramatically different from those calculated previously for models 2, 4, and 5, although there are differences. V_∞ and V_a are quite similar to previous estimates, while K_t is similar except at very low and very high $[\text{Na}]$'s. The $[\text{G}]$ which gives a velocity equal to $V_\infty/2$ is somewhat higher than previous estimates, while estimates of J_m and κ_{Na} are considerably lower.

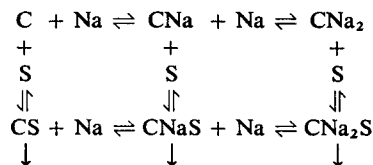
The most significant difference between model 1 and the other models is in fundamental mechanism. The assumptions embodied in this model are as follows:

- (1) there is a fixed total concentration of carrier, $[\text{C}]_t$;
- (2) there is no fixed order of combination of sodium and GABA with the carrier;
- (3) reactions of sodium and GABA with the carrier may be treated as equilibria;
- (4) translocation may occur as CNaG or as CNa_2G .

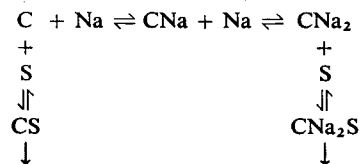
From this model, and from the computed fit of the data to the model, we arrive at the following conclusions:

- (1) uptake is totally sodium dependent;
- (2) since translocation can take place as CNaG and as CNa_2G , the coupling ratio is either one or two;
- (3) at high $[\text{Na}]$, uptake takes place primarily through CNa_2G , while at low $[\text{Na}]$, a majority of uptake is through CNaG ;
- (4) the rate constant for the CNaG species is much higher than that for the CNa_2G species, which implies that the di-sodium form moves through the membrane with much more difficulty than does the mono-sodium form;
- (5) plots of uptake through CNa_2G as a function of $[\text{Na}]$ are sigmoidal in shape, while such plots for uptake through CNaG pass through maximal values and decline with further increases in $[\text{Na}]$;
- (6) as a consequence of (5), plots of total uptake versus $[\text{Na}]$ pass through maximal values and decline with further increases in $[\text{Na}]$. Thus increasing $[\text{Na}]$ above certain critical levels results in inhibition of uptake—the $[\text{Na}]$ at which this occurs depends on the GABA concentration. However, at physiological $[\text{Na}]$, velocity is close to the optimal value for all $[\text{G}]$'s;
- (7) V_a , the apparent maximal velocity, is sodium dependent. V_a increases with $[\text{Na}]$, passes through a maximal value, and declines with further increases in $[\text{Na}]$;
- (8) K_t , the apparent Michaelis constant, is sodium dependent. K_t declines with increasing $[\text{Na}]$, which implies that apparent carrier affinity for GABA increases with $[\text{Na}]$. The apparent affinity at physiological $[\text{Na}]$ is 86.8% of the theoretical maximal value.
- (9) J_m , rate of uptake at a given $[\text{G}]$ and infinite $[\text{Na}]$ is hyperbolically related to the GABA concentration;
- (10) κ_{Na} , the $[\text{Na}]$ which gives $J_m/2$, is dependent on the GABA concentration; κ_{Na} declines as $[\text{G}]$ increases, which implies that the apparent affinity of the carrier for sodium increases as the GABA concentration increases.

The sodium dependence of the uptake of two other substrates, glutamic acid and choline, has also been studied in cortical synaptosomes (Wheeler, 1979a,b). In all three cases, the minimal best fit model is a simplification of the following model:



The model for glutamic acid was found to be identical to that discussed here for GABA. For choline, the model could be further simplified as follows:



That certain components of the larger model drop out for GABA and glutamic acid while others drop out for choline may simply reflect the fact that the concentration of each of these components is too low to measurably affect the rate equation. Although these components may in fact not exist, this cannot be proved by the optimization process. It may be that the dissociation constants are such that the concentration of these components is low, even though they are involved in the process of loading the carrier with sodium and substrate.

Sodium-dependent transport mechanisms represent one of the most important means by which organic solutes are transported across the cell membrane, and are present in widely divergent organisms, from marine bacteria to mammals (Schultz and Curran, 1970). It is interesting to speculate that uptake of other substrates in other cells and organisms might take place by a similar process to that found for choline, glutamic acid, and GABA in rat brain synaptosomes.

Acknowledgements

The author would like to thank Mrs Anne L. Hungerford and Mrs Donna W. Clark for their technical assistance.

References

- Lockwood, A. P. M. (1961). 'Ringer' solutions and some notes on the physiological basis of their ionic composition. *Comp. Biochem. Physiol.* **2**, 241-289.
- Martin, D. L. and Smith, A. A. (1972). Ions and the transport of gamma-aminobutyric acid by synaptosomes. *J. Neurochem.* **19**, 841-855.
- Nelder, J. A. and Mead, R. (1965). A simplex method for function optimization. *Comput. J.* **7**, 308-315.

- Ryan, L. D. and Roskoski, R. Jr. (1977). Net uptake of gamma-aminobutyric acid by a high affinity synaptosomal transport system. *J. Pharmacol. Exptl. Therapeutics* **200**, 285-291.
- Schultz, S. G. and Curran, P. F. (1970). Coupled transport of sodium and organic solutes. *Physiol. Rev.* **50**, 637-718.
- Wheeler, D. D. (1978). Some problems inherent in transport studies in synaptosomes. *J. Neurochem.* **30**, 109-120.
- Wheeler, D. D. (1979a). A model of high affinity choline transport in rat cortical synaptosomes. *J. Neurochem.* **32**, 1197-1213.
- Wheeler, D. D. (1979b). A model of high affinity glutamic acid transport by rat cortical synaptosomes—a refinement of the originally proposed model. *J. Neurochem.* **33**, 883-894.
- Wheeler, D. D. (1980a). A model for GABA and glutamic acid transport by cortical synaptosomes. *Pharmacology* **21**, 141-152.
- Wheeler, D. D. (1980b). Sodium dependence of GABA transport in rat hypothalamic synaptosomes. *J. Neuroscience Res.* **5**, 323-337.
- Wheeler, D. D. and Hollingsworth, R. G. (1979). A model of GABA transport by cortical synaptosomes from the Long-Evans rat. *J. Neuroscience Res.* **4**, 265-289.