A Comparison of the Rate Equations, Kinetic Parameters, and Activation Energies for the Initial Uptake of L-Lysine, L-Valine, γ-Aminobutyric Acid, and α-Aminoisobutyric Acid by Mouse Brain Slices*

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Summary. At substrate concentrations, in medium, of 0.2 to 20 mM and at temperatures of 25 and 37 °C, the initial concentrative influx of the amino acids L-lysine (30 and 37 °C), L-valine, and γ-aminobutyric acid into incubated mouse-cerebrum slices follows the rate equation for the initial influx of α-aminoisobutyric acid (Cohen, J. Physiol. 228:105, 1973),

\[ v = \frac{V_{\text{max}}}{1 + K_{i}S} + k_{u}S. \]

Kinetic constants at 37 °C are: 

- For L-lysine: 
  \[ V_{\text{max}} = 0.089 \mu\text{moles/g final wet wt of slices, min}, \]
  \[ K_{i} = 0.69 \text{mM}, \]
  \[ k_{u} = 0.037 \mu\text{moles/g final wet wt, mm-substrate, min for L-lysine}; \]

- For L-valine: 
  \[ V_{\text{max}} = 0.60, \]
  \[ K_{i} = 1.30, \]
  \[ k_{u} = 0.067 \text{ for L-valine}; \]

- For γ-aminobutyric acid: 
  \[ V_{\text{max}} = 1.71, \]
  \[ K_{i} = 1.58, \]
  \[ k_{u} = 0.094 \text{ for γ-aminobutyric acid}. \]

The linear term, \( k_{u}S \), is due to an unsaturable process of concentrative uptake, not diffusion. Comparison of temperature coefficients reveals a “reference” pattern for typical low affinity transport of amino acids into brain slices. Its characteristics are: Activation energies associated with \( V_{\text{max}} \) and \( k_{u} \) are in range 14 to 20 kcal/mole; \( K_{i} \) varies only slightly with temperature. L-Lysine and α-aminoisobutyric acid fit this pattern; L-valine and γ-aminobutyric acid deviate in part. The Akedo-Christensen plot (J. Biol. Chem. 237:118, 1962) does not distinguish between the rate equation \( v = \frac{V_{\text{max}}}{1 + K_{i}S} + k_{u}S \) for saturable uptake plus first-order unsaturable concentrative uptake, and the rate equation \( v = \frac{V_{\text{max}}}{(1 + K_{i}/S)} + k_{D}(S-S_{i}) \) for saturable uptake plus first-order non-concentrative "passive diffusion".

The transport of amino acids to and from central nervous tissue has been studied extensively for a number of years. (See Neame, 1961; Cohen & Lajtha, 1972 for reviews.) Little is known about the detailed rate equations and activation energies for these processes in vitro, and some of the published studies are questionable. Recently, the author (Cohen, 1973a) found that the influx of the nonmetabolizable amino acid analog, α-aminoisobutyric acid,
acid, follows a rate equation (Eq. 1) that indicates two parallel components, one saturable and one unsaturable, and he measured the three parameters, $V_{\text{max}}$, $K_I$, and $k_u$, in the rate equation and their temperature coefficients. This paper presents a continuation of those studies which was specifically undertaken to answer the following questions: 1) To what extent are the results valid for natural, metabolizable L-$\alpha$-amino acids? and 2) How do the rate equation and temperature coefficients for the uptake of the putative neurotransmitter, GABA, compare with those for nonneurotransmitter $\alpha$-amino acids despite the differences in structure and function, and despite the evidence that they are transported by different carriers (Cohen & Lajtha, 1972)? Since AIB is believed to be carried by the transport system for small neutral amino acids (Cohen & Lajtha, 1972), the $\alpha$-amino acids L-lysine (large basic amino acids), and L-valine (large neutral amino acids) were chosen for this study. To allow valid comparisons with the other amino acids the low-affinity transport of GABA was studied.

**Materials and Methods**

The initial rate of influx of the three amino acids was measured following the procedure used to study the kinetics of AIB influx (Cohen, 1973a). Six- to nine-week old Swiss mice from the Institute colony were decapitated and the brain rapidly removed. The olfactory bulbs and underlying white matter were trimmed from the cerebral hemispheres, which were then immersed in chilled medium for a few seconds. The tissue was removed from the medium, blotted, and nominally 0.37-mm thick slices cut with the calibrated (Cohen, 1974) McIlwain-Buddle (1953) tissue chopper as described by Blasberg and Lajtha (1965). (Males were used almost exclusively even though no difference between the sexes was observed in the earlier study.) Slices from one hemisphere (about 125 to 150 mg) were placed in 4.5 ml of oxygenated, substrate-free medium at temperature in a 25-ml stoppered erlenmeyer flask, and preincubated for 30 min in a thermostatted, reciprocating water bath. One-half ml of medium at temperature containing $^{14}$C-labeled substrate with carrier at 10 times the desired concentration was quickly added and incubation continued for a predetermined interval. The tissue was rapidly filtered off with suction. The resulting tissue pellet was frozen in dry ice, weighed, homogenized in 5 per cent (w/w) perchloric acid, and the concentration of substrate (actually acid-soluble $^{14}$C-labeled substances) determined by liquid scintillation counting as described previously (Cohen, Blasberg, Levi & Lajtha, 1968; Cohen, Stampleman & Lajtha, 1970). The range of incubation periods in which uptake increased linearly with time (Fig. 1) was determined for 0.2 and 20, or 0.2, 2 and 20 mM substrate. The rate of net influx was calculated from the slope of this linear region. At intermediate concentrations, tissue was incubated with substrate for either of two periods within the linear region (e.g. 6 and 14 min for L-lysine at 30 °C); the rate of net influx during this period was calculated. These procedures were adopted to eliminate the contribution of influx

1 *Abbreviations*: AIB, $\alpha$-aminoisobutyric acid; GABA, $\gamma$-aminobutyric acid; HEPES, N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid.