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KINETICS OF COMPETITIVE INHIBITION OF NEUTRAL AMINO ACID TRANSPORT ACROSS THE BLOOD–BRAIN BARRIER

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Abstract—The transport of tryptophan across the blood–brain barrier is used as a specific example of a general approach by which rates of amino acid influx into brain may be predicted from existing concentrations of amino acids in plasma. The kinetics of inhibition of [^{14}C]tryptophan transport by four natural neutral amino acids (phenylalanine, leucine, methionine, and valine) and one synthetic amino acid (α-methyl tyrosine) is studied with a tissue-sampling, single injection technique in the barbiturate-anesthetized rat. The equality of the K_i (determined from cross-inhibition studies) and the K_m (determined from auto-inhibition data) for neutral amino acid transport indicate that these amino acids compete for a single transport site in accordance with the kinetics of competitive inhibition. Based on equations derived for competitive inhibition, apparent K_m values are computed for the essential neutral amino acids from known data on amino acid transport K_m and plasma concentrations. The apparent K_m values make possible predictions of the *in vivo* rates of amino acid influx into brain based on given plasma amino acid concentrations. Finally, a method is presented for determining transport constants from saturation data obtained with single injection techniques.

THE AVAILABILITY of essential amino acids in brain is emerging as an important mechanism by which many pathways of cerebral metabolism are regulated (PARDRIDGE, in press). Amino acid supply in brain has been shown to influence the rate of synthesis of several putative neurotransmitters (FERNSTROM & WURTMAN, 1972; SCHWARTZ *et al.*, 1972; WURTMAN *et al.*, 1974). Cerebral protein synthesis may also be substrate-limited as LAJTHA (1974) and PRATT (1976) have emphasized the close approximation of rates of amino acid influx into brain and rates of amino acid incorporation into protein.

Since the rates of transport of circulating essential amino acids into brain are of rate-limiting significance to brain metabolism, it would be advantageous to have a method by which rates of amino acid influx into the CNS may be predicted on the basis of existing plasma amino acid concentrations. The present study utilizes the transport of circulating tryptophan into brain as a specific example of a general approach by which the rates of transport of amino acids into brain may be estimated from amino acid levels in plasma. It will be shown that predicted rates of amino acid influx compare closely with experimentally observed values. A method will also be presented for the determination of the K_m of substrate transport across the blood–brain barrier (BBB) from saturation data obtained with single injection techniques.

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Abbreviations used: BBB, blood–brain barrier; BUI, brain uptake index; K_m(app), apparent K_m.

METHODS

Influx measurements. The influx into brain of a tracer concentration of L-tryptophan (side chain-3-14C) (New England Nuclear, Boston, MA) was measured with a tissue-sampling, single injection technique developed by OLDENDORF (1970) for the study of BBB transport of radiolabelled compounds. Sprague–Dawley rats, 275–350 g, were obtained from Charles–River Labs (Wilmington, MA), housed two per cage, and fed *ad lib.* until the morning of the experiment. An injection solution was prepared containing 0.25 μCi of [^{14}C]tryptophan and 1.25 μCi of [^{3}H]water (used as a highly diffusable reference of brain uptake) in 0.2 ml of Ringer's solution buffered to pH of 7.4 (5 mM-HEPES, Sigma Chemical Co., St. Louis, MO). The test solution was rapidly (less than 0.5 s) injected into a common carotid artery of the anesthetized rat (Nembutal, 45 mg/kg). Circulation was terminated by decapitation 15 s after injection, a time sufficient for the bolus to make a single passage of the brain circulation (OLDENDORF, 1970). Brain tissue rostral to the midbrain and ipsilateral to the injection was analyzed by double isotope liquid scintillation counting as was a sample of the injection solution. A brain uptake index (BUI) was determined from the ratio of d.p.m. for the ^{14}C to ^{3}H isotope in brain tissue divided by the same ratio in the injection solution. By definition, the BUI = E/E_{HOH}, where E is the fractional extraction of unidirectional influx of [^{14}C]tryptophan lost from blood to brain on a single circulatory pass, and E_{HOH} is the fractional extraction of brain clearance of the [^{3}H]water reference 15 s after carotid injection.

Efflux measurements. The BUI provides a reliable index of the fractional extraction of unidirectional influx of [^{14}C]tryptophan into brain if efflux of label is shown to be negligible during the 15 s circulation period. The rate of efflux of [^{14}C]tryptophan was measured by prolonging the circulation time from 15 s to 4 min. The fractional extraction of [^{14}C]tryptophan was determined by multi-

plying the BUI times the previously reported extraction data for the [^{3}H]water reference at each respective time interval (PARDRIDGE & OLDENDORF, 1975*a*).

Albumin binding studies. Since tryptophan is the only amino acid bound to albumin, the effects of albumin binding on the transport of tryptophan into brain was studied. Approximately 10 ml of blood was obtained by aortic puncture of the anesthetized rat. Four to five ml of rat serum was decanted after centrifuging the coagulated blood for 45 min at 2500 rev./min at 4°C. The serum was placed in standard dialysis tubing and dialyzed against 500 ml of buffered Ringer's solution for 2 h at room temperature. The dialysis was repeated with new Ringer's solution overnight at 4°C. The dialyzed serum was sterilized by passage through a 0.2 μm millipore-filter and then stored at −20°C. The effects of dialyzed rat serum on tryptophan transport was also compared to commercial albumin. In a few experiments the final injection solution was made 3% fatty acid free-bovine albumin (Sigma Chemical Co.).

Competition studies. The effects of competition by other neutral amino acids on the influx of a tracer quantity (0.02 mM) of [^{14}C]tryptophan was determined by adding 0.1, 0.5, 1.0, and 4.0 mM concentrations of unlabelled amino acid to the injection solution. A double reciprocal plot of the inhibition data yielded the K_i (mM) of amino acid transport, i.e. the concentration at which 50% inhibition is observed of the saturable component of [^{14}C]tryptophan transport. The basis for the type of double reciprocal plot used in these and other experiments (PARDRIDGE & CONNOR, 1973; PARDRIDGE & OLDENDORF, 1975*a*, *b*) is explained in the Appendix.

RESULTS

Influx and efflux of [^{14}C]*tryptophan*

The BUI ± S.E.M. of a tracer concentration (0.02 mM) of [^{14}C]tryptophan is 33.2 ± 1.6%. The relationship between the BUI of [^{14}C]tryptophan versus the circulation time after bolus injection is presented in Fig. 1A. The BUI increases with time since the rate of efflux of the water reference is faster than the efflux of tryptophan. The rate of washout of [^{14}C]tryptophan is given in Fig. 1B. These data show that there is no loss of labelled amino acid for at least 2 min after transport into brain. Therefore, the BUI of tryptophan is a reliable index of the fractional extraction of unidirectional influx of amino acid into brain.

Albumin binding

The data in Table 1 indicate that when the final injection solution is made 67% dialyzed ratserum, a statistically significant 15% depression of the BUI of tryptophan is observed. Furthermore, a statistically significant 31% decrease in the BUI of tryptophan is observed if the final injection solution is made 3% fatty acid free-bovine albumin. Neither rat serum or bovine albumin alter the BUI of [^{14}C]leucine.

Amino acid competition

If unlabelled neutral amino acid is added to the injection solution, a varying decrease in the BUI of

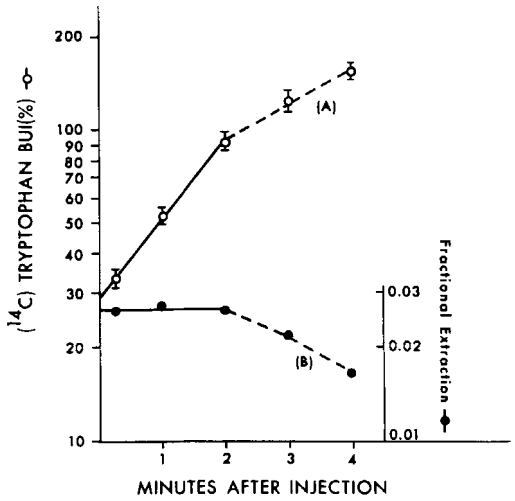

FIG. 1. (A) The BUI of a tracer concentration (0.02 mM) of [^{14}C]tryptophan is plotted against the time after carotid injection. Means ± S.E.M. are based on data from 3 to 5 rats. (B) The fractional extraction of [^{14}C]tryptophan clearance by brain after common carotid injection versus the circulation time. The dashed component of the efflux curve represents exodus from brain of either [^{14}C]tryptophan or an unknown metabolite.

[^{14}C]tryptophan is observed depending on the concentration of amino acid (Table 2). The effects of increasing concentrations of unlabelled phenylalanine or valine on the brain uptake of [^{14}C]tryptophan is depicted in Fig. 2A. Double reciprocal plots of these data (Fig. 2B) provide the respective K_i. The K_i of four natural neutral amino acids (phenylalanine, leucine, methionine, and valine) and one synthetic neutral amino acid, α-methyl tryosine, are listed in Table 3. The K_i values, determined from cross-inhibition studies, compare closely with the individual K_m values reported previously (Table 3). The latter were determined from auto-inhibition data (PARDRIDGE & OLDENDORF, 1975*b*). The approximation of K_m and K_i data indicate that the mechanism of cross-inhibition of tryptophan transport by other neutral amino acids is competitive inhibition (CHRISTENSEN, 1969).

TABLE 1. EFFECTS OF ALBUMIN ON BRAIN UPTAKE OF TRYPTOPHAN AND LEUCINE

Injection vehicle	Brain uptake index (%)*	
	[^{14}C]Tryptophan (0.02 mM)†	[^{14}C]Leucine (0.004 mM)†
Ringer's solution	33.2 ± 1.1	46.1 ± 0.9
67% Rat serum (dialyzed)	28.3 ± 1.2‡	48.1 ± 2.1§
3% Bovine albumin (fatty acid free)	23.0 ± 3.2‡	46.1 ± 3.0§

* Values are means ± S.E.M. based on data from 3 to 5 animals.

† Tracer concentration of labelled amino acid in the injection solution.

‡ $P < 0.05$.

§ n.s.

TABLE 2. NEUTRAL AMINO ACID INHIBITION OF THE BRAIN UPTAKE OF [^{14}C]TRYPTOPHAN

Competing amino acid	[^{14}C]Tryptophan brain uptake index (%)*			
	0.1 mM	0.5 mM	1 mM	4 mM
Phenylalanine	16.8 ± 0.5	7.9 ± 1.0	6.5 ± 1.6	3.6 ± 0.5
Leucine	20.2 ± 0.5	10.3 ± 0.1	8.7 ± 0.4	3.8 ± 0.1
Methionine	23.1 ± 0.8	15.7 ± 0.7	10.1 ± 0.6	5.5 ± 0.2
α-Methyl tyrosine	25.7 ± 0.5	20.7 ± 0.4	14.8 ± 2.6	8.7 ± 0.3
Valine	28.4 ± 1.0	21.5 ± 0.1	18.6 ± 0.7	10.8 ± 0.6

* Values are means ± S.E.M. based on data from 3 to 5 animals. The concentration of [^{14}C]tryptophan in the injection solution was 0.02 mM. The concentration of unlabelled competing amino acid in the injection solution was 0.1, 0.5, 1, and 4 mM, respectively.

Rates of unidirectional influx of neutral amino acids

The rate of unidirectional influx (v) of a circulating amino acid into brain may be estimated from the Michaelis–Menten equation,

$$v = \frac{V_{max}(S)}{K_m(\text{app}) + (S)} \quad (1)$$

where (S) is the plasma amino acid concentration, V_{max} is the maximal transport rate, and K_m(app) is the apparent K_m of transport. The K_m(app) for tryptophan is defined according to the laws of competitive inhibition (CLELAND, 1967):

$$K_m(\text{app})^{trp} = K_m^{trp}\left(1 + \sum\frac{(S)}{K_m}\right). \quad (2)$$

Given the sum of ratios of plasma concentration to absolute transport K_m for each of the competing amino acids, the K_m(app) for tryptophan may be calculated. Plasma amino acid concentrations (BANOS *et al.*, 1973) and absolute transport K_m values (PARDRIDGE & OLDENDORF, 1975*b*) for all the essential amino acids are known for the anesthetized rat. Substitution of these data into equation (2) gives an estimate of the K_m(app) for each of the essential neutral amino acids (Table 4). Substitution of the plasma concentration, V_{max}, and K_m(app) into equation (1) results in predicted rates of amino acid influx into brain (Table 4). A plasma level of 0.004 mM-DOPA was used as a representative therapeutic concentration of this amino acid (BIRKMAYER *et al.*, 1973). The predicted rates of amino acid influx compare favorably with experimentally observed values reported by BANOS *et al.* (1973). A correlation analysis (not shown here) between the predicted and observed rates of amino acid influx in Table 4 gives a correlation coefficient of 0.81. If the phenylalanine data is omitted from the analysis, the correlation coefficient is 0.93.

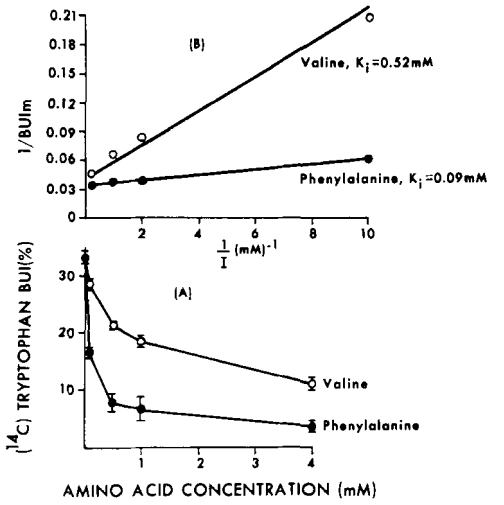

FIG. 2. (A) Inhibition of the influx into brain of [^{14}C]tryptophan (0.02 mM) by increasing concentrations of either unlabelled phenylalanine or valine. Means ± S.E.M. are based on data from 3 to 5 rats. (B) Double reciprocal transformation of the inhibition data. The K_i is determined from the slope/intercept ratio (see Appendix).

TABLE 3. RELATIONSHIP OF K_m AND K_i OF NEUTRAL AMINO ACID TRANSPORT

Amino acid	K_m (mM)*	K_i (mM)†
Phenylalanine	0.12	0.09
Leucine	0.15	0.13
Methionine	0.19	0.16
α-Methyl tyrosine	—	0.31
Valine	0.63	0.52

* From PARDRIDGE & OLDENDORF (1975*b*).
† Obtained from linear transformations of the data in Table 2 (see Appendix).

DISCUSSION

The data presented in this study are an extension of a previous report (PARDRIDGE & OLDENDORF, 1975*b*) in which the kinetic parameters of BBB transport were analyzed as the individual amino acids were delivered to brain isolated in a saline bolus. However, multiple amino acids compete for common transport systems (CHRISTENSEN, 1969). Consequently, the kinetic parameters of amino acid transport into brain from an amino acid mixture in plasma are not equivalent to kinetic constants obtained when amino acids enter brain isolated in saline (PARDRIDGE *et al.*, 1975). Although competitive inhibition of transport does not alter the V_{max}, the K_m of transport is increased according to equation (2). Comparing the K_m(app) data in

TABLE 4. PREDICTED RATES OF AMINO ACID INFLUX INTO BRAIN BASED ON THE KINETIC CONSTANTS OF AMINO ACID TRANSPORT AND PLASMA AMINO ACID LEVELS

Amino acid	Plasma* level (mM)	K_m(app)† (mM)	V_{max}‡ (nmol min^{-1} g^{-1})	v_{pre}§ (nmol min^{-1} g^{-1})
Phenylalanine	0.05	0.45	30	3.0 (6.5)
Leucine	0.10	0.53	33	5.2 (6.2)
Tyrosine	0.09	0.58	46	6.2 (5.3)
Tryptophan	0.10	0.71	33	4.1 (2.5)
Methionine	0.04	0.77	33	1.6 (1.6)
Histidine	0.05	1.1	38	1.6 (2.5)
Isoleucine	0.07	1.3	57	2.9 (1.9)
DOPA	0.004	1.9	64	0.1 (0.1)
Valine	0.14	2.5	49	2.5 (1.8)
Threonine	0.19	3.0	37	2.2 (2.3)
Cycloleucine	—	3.2	55	—

* From BANOS *et al.* (1973) for amino acids other than DOPA, which is from BIRKMAYER *et al.* (1973).

† Calculated from equation (2).

‡ From PARDRIDGE & OLDENDORF (1975*b*).

§ Predicted rates of influx calculated from equation (1) of text. Observed rates of influx are in parentheses from BANOS *et al.* (1973) except for DOPA, which is from DANIEL *et al.* (1976).

Table 4 with absolute K_m values (PARDRIDGE & OLDENDORF, 1975*b*) indicates that the competition between amino acids for a single transport site raises the apparent K_m between 3- and 4-fold. For example, the K_m of phenylalanine transport isolated in solution is 0.12 mM as opposed to a K_m(app) of 0.45 mM when other competing amino acids are present at their normal plasma levels.

The calculation of the K_m(app) for the large, neutral amino acids is based on three assumptions: (i) albumin-bound tryptophan competes for transport sites, (ii) amino acids compete for a single binding site, i.e. competitive inhibition, and (iii) the affinity of the small, neutral amino acids for transport sites is low enough to only modestly influence the K_m(app).

Tryptophan is the only amino acid bound to albumin (MCMENAMY *et al.*, 1961). MADRAS *et al.* (1974) have suggested that both bound and free tryptophan compete for brain transport sites based on the assumption that the affinity of albumin binding sites approximates that of the brain transport system. The dissociation constant (K_{diss}) of albumin binding sites may be estimated from the law of mass action, i.e.

$$K_{diss} = \frac{\text{(free tryptophan)(albumin)}}{\text{(albumin bound-tryptophan)}}. \quad (3)$$

Substitution of the data of FERNSTROM *et al.* (1975) for free and bound tryptophan, and an albumin concentration of 0.55 mM (MCMENAMY *et al.*, 1961) into equation (3) indicates that the K_{diss} of albumin binding of tryptophan varies from 0.17 mM to 0.34 mM as the level of free fatty acid changes from 0.4 mM to 1.2 mM. Free fatty acid is known to decrease the affinity of albumin for tryptophan (MADRAS *et al.*, 1974). Since the K_{diss} of albumin binding of tryptophan approximates the K_m(app), 0.71 mM (Table 4), of BBB transport of tryptophan, a substantial fraction of protein bound amino acid competes for BBB transport sites. The data in Table 1 further suggest that tryptophan may be stripped off albumin by barrier transport systems. Although 80–90% of plasma tryptophan is bound by albumin (MADRAS *et al.*, 1974), the BUI of [^{14}C]tryptophan in the presence of dialyzed rat serum is depressed by only 15%. Dialyzed serum has no effect on the BUI of [^{14}C]leucine (Table 1) of [^{14}C]methionine (OLDENDORF, 1971), which is consistent with the lack of protein binding of these amino acids. The greater depression of the BUI of [^{14}C]tryptophan by fatty acid free albumin (Table 1) is consistent with the inverse relationship between the level of plasma free fatty acid and the affinity of albumin for tryptophan. YUWILER *et al.* (1976) have shown that, depending on the amount of free fatty acid in plasma, as much as 50%–90% of protein bound tryptophan may be stripped off albumin during a single circulatory pass of brain. Therefore, the data obtained by several techniques supports the observations of MADRAS *et al.* (1974) that both free and bound plasma tryptophan compete for brain transport sites.

The use of equation (2) is dependent on the premise that the inhibition of tryptophan transport by other neutral amino acids is due to competition at a single binding site, i.e. competitive inhibition. In addition to the data in Table 3, other studies have reported evidence supporting the validity of equation (2). BETZ *et al.* (1975) and BANOS *et al.* (1974) have shown that the branched chain and basic amino acids, respectively, compete for transport sites according to competitive inhibition. WADE & KATZMAN (1975*a*) have also shown that DOPA and 3-*O*-methyl DOPA compete for a common binding site. *O*-methylation increases the affinity of the BBB for aromatic amino acids as the K_m of 3-*O*-methyl DOPA transport is 0.13 mM as opposed to the K_m for DOPA, 0.34 mM

(WADE & KATZMAN, 1975*a*). Conversely α-methylation appears to lower the carrier affinity for aromatic amino acids. The K_i for α-methyl tyrosine, 0.31 mM (Table 3), is two-fold the K_m, 0.16 mM, of tyrosine transport (PARDRIDGE & OLDENDORF, 1975*b*).

The K_m(app) data in Table 4 do not account for the small but measurable affinity of the non-essential amino acids for the BBB neutral amino acid transport system (OLDENDORF, 1971). However, the BUI of the small neutral amino acids is less than 10% (OLDENDORF, 1971), which suggests that the K_m of transport of these amino acids is greater than 1 mM (PARDRIDGE *et al.*, 1975). Substitution of hypothetical K_m values for the small neutral amino acids (based on their reported BUI) into equation (2) indicates that the competition effect by these amino acids on the K_m(app) values in Table 4 would not raise the K_m(app) by more than 10%.

The small neutral amino acids are usually transported into cells by the alanine (A) system (CHRISTENSEN, 1969). Large neutral amino acids enter cells primarily via the leucine (L) system (CHRISTENSEN, 1969), although there is much overlap of amino acid avidity for the two systems (CHRISTENSEN, 1973). WADE & KATZMAN (1975*b*) have recently reported cogent evidence that the A-system is not operative in BBB transport. Other workers have also suggested the A-system does not function in barrier transport of amino acids (SCHAIN & WATANABE, 1972; YUDILEVICH *et al.*, 1972). The equivalence of the K_m and K_i values in Table 3 further suggests the latency of the A-system (CHRISTENSEN, 1969). The significance of an inactive A-system in barrier transport of amino acids is two-fold. Firstly, since BBB amino acid transport is mediated only by the L-system, transport of amino acids into brain is sodium-independent and equilibrative (CHRISTENSEN, 1969). The lack of concentrative transport of amino acids across the BBB is one mechanism by which CSF levels of amino acids (PERRY *et al.*, 1975) are maintained at a low level. Secondly, since the A-system is the amino acid transport system that is insulin-inducible (RIGGS & MCKIRAHAN, 1973), barrier transport of amino acids is probably insulin-insensitive.

FERNSTROM & WURTMAN (1972) have demonstrated the exquisite sensitivity of neutral amino acid influx into brain to physiological changes in amino acid plasma levels *in vivo*. The approximation of BBB transport K_m by plasma amino acid levels forms the basis underlying the marked effects of competition on amino acid influx into brain. Competitive inhibition of amino acid transport has been shown to occur in a variety of tissues *in vitro* and is an important criterion for the presence of carrier-mediated transport. However, the K_m of large, neutral amino acid transport in intestine (LARSEN *et al.*, 1964), liver (PARDRIDGE & JEFFERSON, 1975), kidney (LINGARD *et al.*, 1973), and erythrocyte (WINTER & CHRISTENSEN, 1964) is greater than 1 mM or at least 10-fold BBB K_m values and at least 10-fold plasma amino acid levels. Equation (2) predicts that the effects of competition on amino acid transport are not important until substrate levels approach the K_m value. Therefore, competition at amino acid transport sites, within the physiological range of plasma amino acid levels, probably does not occur in tissues other than brain. The biological advantage of high K_m amino acid transport is related to Kreb's observation (KREBS, 1972) that amino acid excesses are quickly degraded in the tissues because amino acid pathways are governed by high K_m processes and are, therefore, never saturated. Conversely, amino acid pathways in brain are governed by low K_m processes. Therefore, competition at transport sites readily occurs and the brain is acutely alerted to changes in plasma amino acid composition. ASHLEY & ANDERSON (1975) have recently shown that the neuroregulation of protein intake is a function of the ratio of plasma tryptophan to sum of competing neutral amino acids (Trp/ΣNAA). The Trp/ΣNAA ratio is derived from equation (2) and reflects the brain's unusual sensitivity to the effects of competition for amino acid transport sites.

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APPENDIX

The rate of unidirectional influx (v) of [^{14}C]tryptophan into brain is given by the relationship, $v = (E)(F)(S_t)$, where E is the fractional extraction of unidirectional influx, F is the rate of cerebral blood flow, and S_t is the final tracer concentration of [^{14}C]tryptophan in the injection solution. Alternatively, $v = (\mathrm{BUI})(E_{\mathrm{HOH}})(F)(S_t)$. Since E_{HOH}, F, and S_t are uniform for all injections, BUI is directly proportional to v.

The components of [^{14}C]tryptophan influx in the presence of unlabelled competing amino acid (I) are presented in Fig. 3 as a modification of the method of INUI & CHRISTENSEN (1966). The saturable component of [^{14}C]tryptophan transport (v_o) is given by,

$$v_0 = \frac{V_{\max}(S_t)}{K_m + S_t}.$$

The component of [^{14}C]tryptophan transport that is subject to competitive inhibition by unlabelled amino acid is given by,

$$v_i = \frac{V_{\max}(S_t)}{K_m(1 + \mathrm{I}/K_i) + S_t}.$$

The non-saturable component, 'a', of [^{14}C]tryptophan transport may be eliminated from the computation of the transport constant by setting $\mathrm{BUI}_m = \mathrm{BUI}_o - \mathrm{BUI}_i$, where $\mathrm{BUI}_o = (v_o + a)$ and $\mathrm{BUI}_i = (v_i + a)$ (Fig. 3). Therefore, $\mathrm{BUI}_m = (v_o - v_i)$ and

$$\mathrm{BUI}_m = \frac{V_{\max} K_m S_t \mathrm{I}}{K_i(K_m + S_t)(K_m + K_m\mathrm{I}/K_i + S_t)},$$

$$1/\mathrm{BUI}_m = \frac{K_i(K_m + S_t)^2}{V_{\max} K_m S_t}\frac{1}{\mathrm{I}} + \frac{K_m + S_t}{V_{\max} S_t},$$

$$1/\mathrm{BUI}_m = (\mathrm{slope})(1/\mathrm{I}) + (\mathrm{intercept}),$$

$$\frac{(\mathrm{slope})}{(\mathrm{intercept})} = K_i(1 + S_t/K_m) = K_i,$$

$$\text{if} \quad K_i \gg (K_i/K_m)(S_t).$$

If the tracer [^{14}C]tryptophan competes for transport sites with unlabelled tryptophan, the 'K_i' of unlabelled tryptophan is actually the transport K_m and the (slope)/(intercept) ratio is equal to $(K_m + S_t)$. In either the case of determining the K_m or the K_i of amino acid transport, the (slope)/(intercept) ratio does not approximate the transport constant unless S_t is several-fold less than the transport constant. If $S_t = 0.02$ mM and $K_m = 0.20$ mM, then the (slope)/(intercept) ratio will overestimate the K_m by 10%. Therefore, the concentration of labelled amino acid in the injection solution will not approach 'tracer' values until S_t is at least 10-fold less than K_m.

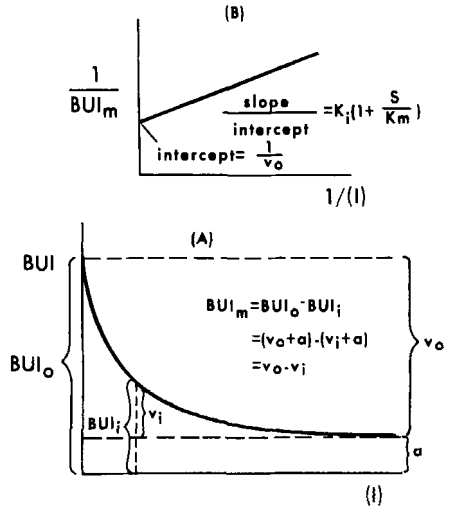

FIG. 3. (A) Model curve of the inhibition of [^{14}C]tryptophan influx into brain by competing unlabelled amino acid. BUI_o reflects the brain uptake of a tracer concentration of labelled tryptophan in the absence of competing amino acid; BUI_i represents the brain uptake of [^{14}C]tryptophan in the presence of a given concentration of competing amino acid (I); BUI_m, v_o, v_i, and 'a' are defined in the text. (B) Lineweaver–Burk plot of the reciprocals of BUI_m and I. The transport constant is obtained from the slope/intercept ratio as defined in the text.