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Blood–Brain Barrier Transport of 1-Aminocyclohexanecarboxylic Acid, a Nonmetabolizable Amino Acid for In Vivo Studies of Brain Transport

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Abstract: Regional transport of 1-aminocyclohexanecarboxylic acid (ACHC), a nonmetabolizable amino acid, across the blood–brain barrier was studied in pentobarbital-anesthetized rats using an in situ brain perfusion technique. The concentration dependence of influx was best described by a model with a saturable and a nonsaturable component. Best-fit values for the kinetic constants of the frontal cortex equaled 9.7×10^{-4} μmol/s/g for V_{max}, 0.054 μmol/ml for K_m, and 1.0×10^{-4} ml/s/g for K_D in the absence of competing amino acids. Saturable influx could be reduced by >85% by either L-phenylalanine or 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid, consistent with transport by the cerebrovascular neutral amino acid transport system. The transport K_m for ACHC was one-fifth that for the more commonly used homologue, 1-aminocyclopentanecarboxylic acid, and was similar to values for several natural amino acids, such as L-methionine, L-isoleucine, and L-tyrosine. The results indicate that ACHC may be a useful probe for in vivo studies of amino acid transport into brain. **Key Words:** Amino acid—Blood–brain barrier—Transport—Capillary—Brain—Rat. **Aoyagi M. et al.** Blood–brain barrier transport of 1-aminocyclohexanecarboxylic acid, a nonmetabolizable amino acid for in vivo studies of brain transport. *J. Neurochem.* **50,** 1220–1226 (1988).

Amino acids are transported from plasma to brain by specific saturable carriers at the cerebral capillaries of the blood–brain barrier (Oldendorf, 1971; Oldendorf and Szabo, 1976). Regional transport rates can be measured in vivo in humans using positron emission tomography or in experimental animals using quantitative autoradiography. In most studies, labeled forms of natural amino acids, such as L-methionine or L-leucine, have been used (Comar et al., 1981; Mans et al., 1982; Phelps et al., 1985; Bergstrom et al., 1987). However, natural amino acids have limitations in transport studies because subsequent to uptake, they are metabolized and incorporated into proteins. These processes can lead to errors in measured influx rates and prevent accurate determination of efflux rates.

To overcome these limitations, synthetic amino acids have been developed that are neither metabolized nor incorporated into proteins. One such amino acid, 1-aminocyclopentanecarboxylic acid (ACPC), has been used extensively to study neutral amino acid (NAA) transport in peripheral tissues (Oxender and Christensen, 1963; Christensen, 1973). ACPC is transported by the NAA carrier at the blood–brain barrier (Pardridge and Oldendorf, 1975). However, the carrier affinity for the amino acid is low, approximately one-tenth that of L-leucine (Smith and Takasato, 1986).

Washburn et al. (1981) have suggested that a homologue based on a cyclohexane rather than a cyclopentane ring, 1-aminocyclohexanecarboxylic acid (ACHC), may have greater affinity than ACPC for the cerebrovascular NAA transport system. ACHC, like ACPC, is neither metabolized nor incorporated into tissue proteins (Christensen and Jones, 1962). We therefore thought it of interest to examine the

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Abbreviations used: ACBC, 1-aminocyclobutanecarboxylic acid; ACHC, 1-aminocyclohexanecarboxylic acid; ACPC, 1-aminocyclopentanecarboxylic acid; BCH, 2-aminobicyclo[2.2.1]-heptane-2-carboxylic acid; MeAIB, methylaminoisobutyric acid; NAA, neutral amino acid.

transport kinetics of ACHC at the blood–brain barrier to determine whether it would indeed be a preferable tracer for in vivo studies of cerebrovascular NAA transport. If so, it may prove useful for studies in humans using positron emission tomography (Comar et al., 1981; Bergstrom et al., 1987).

MATERIALS AND METHODS

Materials

[*carboxyl*-^{14}C]ACHC (sp act 377 μCi/mg) was prepared from K^{14}CN and cyclohexanone using a modified Bucherer–Strecker synthesis followed by purification with ion-exchange chromatography (Hayes et al., 1978; Washburn et al., 1979). Radiochemical purity was verified by TLC on silica gel with butanol/water/acetic acid (100:10:5 vol/vol) as solvent. Unlabeled ACHC and 1-aminocyclobutanecarboxylic acid (ACBC) were synthesized and purified as previously described by Connors and Ross (1960). Other amino acids were obtained from Sigma Chemical (St. Louis, MO, U.S.A.). [*N-methyl*-^{3}H]Diazepam and [*methoxy*-^{3}H]inulin were purchased from Amersham–Searle (Arlington Heights, IL, U.S.A.) and New England Nuclear (Boston, MA, U.S.A.), respectively.

Determination of ACHC influx

[^{14}C]ACHC transport across the blood–brain barrier was measured with the in situ brain perfusion technique of Takasato et al. (1984). This procedure allows accurate measurements of influx and complete control of perfusate composition (Smith et al., 1985; Momma et al., 1987).

All experiments used adult male rats (Sprague–Dawley strain), weighing 240–350 g, that were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). In each rat, the right external carotid artery was catheterized for retrograde infusion, and the right pterygopalatine, occipital, and superior thyroid arteries were coagulated and cut. Rectal temperature was maintained at 37 ± 0.5°C with a heat lamp.

To start the perfusion, the right common carotid artery was ligated, and then perfusion fluid was infused into the external carotid artery at 5 ml/min with a pump. In most experiments, the perfusate consisted of HCO_3-buffered physiological saline (pH 7.4, 37°C; Momma et al., 1987) that contained 0.08 μCi/ml of [^{14}C]ACHC, 0.58 μCi/ml of [^{3}H]inulin to measure intravascular volume, and 0–10 μmol/ml of unlabeled amino acid. However, in some experiments a plasma perfusate was used to determine [^{14}C]-ACHC uptake in the presence of normal physiological concentrations of competing amino acids. Plasma for perfusion was obtained from heparinized donor rats on the morning of the experiment. Cerebral perfusion fluid flow was measured in separate animals as described by Takasato et al. (1984).

Twenty seconds after the start of the perfusion, the animal was killed by decapitation and the pump was turned off. Following removal of the brain from the skull, samples from six brain regions were dissected from the right cerebral hemisphere, as previously described by Smith et al. (1985). The tissue samples along with a 20-μl aliquot of perfusion fluid were weighed, digested in 1 *M* piperidine, and prepared for counting by addition of scintillation cocktail. Dual-label counting was performed with a Beckman LS6800 liquid scintillation counter (Beckman Instruments, Fullerton, CA, U.S.A.). Amino acid concentrations in plasma were determined by HPLC (Smith et al., 1985).

Calculations

Unidirectional ACHC influx (J_{in}) into brain was calculated with the following equation

$$J_{in} = [q^*_{br}/C^*_{pf}T]C_{pf} \quad (1)$$

where q^*_{br} = parenchymal brain content of [^{14}C]ACHC at the time of death, C^*_{pf} = perfusate concentration of [^{14}C]-ACHC, C_{pf} = perfusate concentration of ACHC, and T = net perfusion time (Smith et al., 1985). To ensure unidirectional uptake kinetics, perfusion time was limited to 20 s in all experiments (Momma et al., 1987). The value for q^*_{br} was obtained from the measured brain content of [^{14}C]-ACHC (q^*_{tot}) by subtraction of residual intravascular tracer as

$$q^*_{br} = q^*_{tot} - V_v C^*_{pf} \quad (2)$$

where V_v is the brain vascular volume. V_v was measured with [^{3}H]inulin and averaged 0.76 ± 0.03 × 10^{-2} ml/g (mean ± SEM, n = 26) in the six brain regions. The value for q^*_{br} was not corrected for residual tracer in the brain capillary endothelium. [^{14}C]ACHC influx was calculated as q^*_{br}/T.

The concentration dependence of amino acid transport across the blood–brain barrier can be described by a model with a saturable and a nonsaturable component (Pardridge, 1983). In the absence of competing amino acids, J_{in} is given as

$$J_{in} = \frac{V_{max}\bar{C}_{cap}}{K_m + \bar{C}_{cap}} + K_d\bar{C}_{cap} \quad (3)$$

where V_{max} = maximal transport capacity of the saturable component, K_m = half-saturation concentration, K_D = constant of nonsaturable diffusion, and $\bar{C}_{cap}$ = mean capillary concentration of amino acid. For ACHC, $\bar{C}_{cap}$ was calculated as

$$\bar{C}_{cap} = K_{in}C_{pf}/[-F \ln (1 - K_{in}/F)] \quad (4)$$

where K_{in} is the unidirectional transfer constant for ACHC uptake into brain ($K_{in} = q^*_{br}/C^*_{pf}T$) and F = cerebral perfusion fluid flow. F was measured in separate animals from the uptake of [^{3}H]diazepam (Takasato et al., 1984) and ranged from 8.9 ± 0.4 × 10^{-2} ml/s/g (mean ± SEM, n = 9) in the parietal cortex to 4.6 ± 0.3 × 10^{-2} ml/s/g in the caudate nucleus. Because of the rapid flow rate with the perfusion technique, the difference between $\bar{C}_{cap}$ and C_{pf} was small, averaging only 2% overall with a maximal value of 9%. Values of V_{max}, K_m, and K_D were obtained from the weighted least-squares fit of Eq. 3 to the saline perfusion data, as previously described by Smith et al. (1985) and Momma et al. (1987).

With competing amino acids in the perfusate, the concentration dependence of ACHC influx can be described by an equation similar to Eq. 3 but with K_m(app) in place of K_m. K_m(app) is defined as (Pardridge, 1977)

$$K_m(app) = K_m[1 + \Sigma (\bar{C}_{cap(i)}/K_i)] \quad (5)$$

where $\bar{C}_{cap(i)}$ = mean capillary concentration of each competing amino acid and K_i = respective inhibition constant for that amino acid. K_m(app) was calculated from the ACHC influx from plasma using values of V_{max} and K_D from the saline perfusion data.

K_i values for individual amino acids were determined from their inhibition of ACHC influx into brain. At tracer

ACHC concentration, the ratio of J_{in} in the absence of competing amino acid $[(J_{in})_o]$ to that in the presence of competing amino acid $[(J_{in})_i]$ is given as

$$(J_{in})_o/(J_{in})_i = 1 + \bar{C}_{cap(i)}/K_i \quad (6)$$

K_i was calculated from the $(J_{in})_o/(J_{in})_i$ ratio assuming $\bar{C}_{cap(i)}$ equals the perfusate concentration of competing amino acid.

Statistics

All values are means ± SEM, unless otherwise noted. Differences between means were analyzed for statistical significance at the $p < 0.05$ level using one-way analysis of variance and the Bonferroni multiple comparison test (Miller, 1966).

RESULTS

Saline perfusion

Figure 1 illustrates the relationship between ACHC influx and concentration for one brain region, the frontal cerebral cortex. For this experiment, ACHC was the only amino acid in the saline perfusate. The concentration dependence of influx could be described by a model with a saturable and a nonsaturable component. At concentrations < 0.3 μmol/ml, the nonsaturable component contributed minimally (<10%) to total influx.

V_{max}, K_m, and K_D values were calculated by fitting Eq. 3 to the ACHC influx data using weighted nonlinear least squares. Best-fit values of the parameters are listed in Table 1. These values were calculated using the influx data over the entire concentration range from 0.0001 to 10 μmol/ml. To determine whether the fit of the nonsaturable component (K_D) markedly affected the calculated values for V_{max} or K_m, the transport constants were also calculated using only the flux data at low concentrations (≤1 μmol/ml) where the contribution of the nonsaturable component could be ignored ($K_D = 0$). Best-fit values for V_{max} and K_m using this alternate analysis were within 15% and did not differ significantly from corresponding values in Table 1. For example, V_{max} and K_m equaled 10.8 ± 1.2 × 10^{-4} μmol/s/g and 0.062 ± 0.011 μmol/ml (n = 20), respectively, in the frontal cortex.

There were no significant regional differences in K_m or K_D among the six brain regions (Table 1). However, V_{max} was significantly greater in the frontal and parietal cortices than in the hippocampus, caudate nucleus, or thalamus-hypothalamus.

ACHC influx into brain was inhibited competitively by L-phenylalanine. The concentration dependence of inhibition is shown in Fig. 2 for the frontal cortex. Influx was reduced >85% at a perfusate L-phenylalanine concentration of 0.1 μmol/ml. The calculated K_i equaled 0.012 ± 0.001 μmol/ml (mean ± SEM, n = 12) and did not differ from that in the other five brain regions.

Table 2 summarizes the effects of various amino acids on [^{14}C]ACHC uptake into brain. 2-Aminobicyclo[2.2.1]heptanecarboxylic acid (BCH), the model substrate for the L system (Christensen, 1973), reduced [^{14}C]ACHC influx by ~90% at a concentration of 1 μmol/ml. In contrast, neither methylaminoisobutyric acid (MeAIB), the model substrate for the A system, nor L-lysine, a substrate for the cationic (y^+) amino acid transport system, produced significant inhibition of [^{14}C]ACHC influx. ACPC and ACBC, the smaller homologues of ACHC, produced less inhibition of [^{14}C]ACHC influx at 0.3 μmol/ml than ACHC at the same concentration. Calculated K_i values for ACPC and ACBC were, on average, ~5 and ~35 times greater, respectively, than the K_m for ACHC.

Plasma perfusion

Regional ACHC influx from plasma was 8.8 ± 0.5% (n = 5) of that measured at the same concentration using saline perfusate that did not contain competing amino acids. K_{in} from plasma ranged from 13.1 ± 0.9 × 10^{-4} ml/s/g in the frontal cortex to 10.7 ± 0.9 × 10^{-4} ml/s/g in the hippocampus. Competition for transport increased the mean regional K_m(app) by ~14-fold from 0.053 ± 0.002 to 0.72 ± 0.02 μmol/ml. K_m(app) did not differ significantly among the six brain regions.

Table 3 compares regional ACHC influx rates as measured during plasma perfusion with values predicted using Eqs. 3 and 5 and the measured NAA

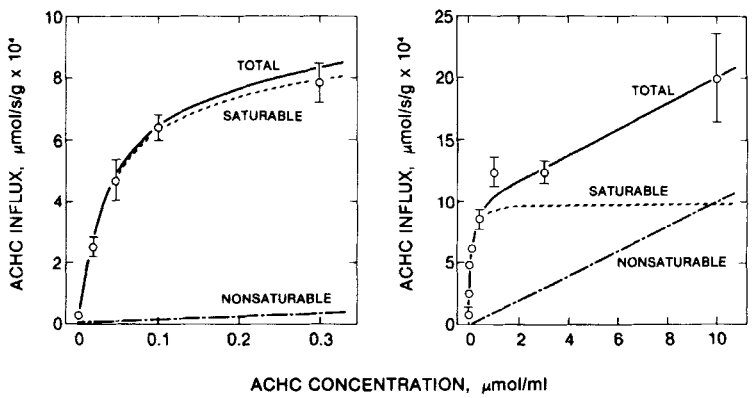

FIG. 1. Unidirectional ACHC influx into frontal cortex as a function of ACHC concentration of saline perfusate. **Left:** Influx versus concentration from 0 to 0.3 μmol/ml. **Right:** Influx versus concentration from 0 to 10 μmol/ml. Data are means ± SEM (bars) values for three or four animals. The curves represent total, saturable, and nonsaturable influxes as predicted by Eq. 3 where V_{max} = 9.7 × 10^{-4} μmol/s/g, K_m = 0.054 μmol/ml, and K_D = 1.0 × 10^{-4} ml/s/g.

TABLE 1. *V_{max}, K_m, and K_D for cerebrovascular transport of ACHC from saline perfusate to brain*

Brain region	V_{max} (μmol/s/g $\times 10^4$)	K_m (μmol/ml)	K_D (ml/s/g $\times 10^4$)
Frontal cortex	9.7 ± 0.7	0.054 ± 0.005	1.0 ± 0.3
Parietal cortex	8.9 ± 0.6	0.048 ± 0.004	1.6 ± 0.3
Occipital cortex	7.7 ± 0.6	0.057 ± 0.008	1.1 ± 0.4
Hippocampus	6.6 ± 0.9[a]	0.056 ± 0.009	1.1 ± 0.5
Caudate nucleus	6.5 ± 0.7[a]	0.049 ± 0.007	1.5 ± 0.3
Thalamus-hypothalamus	6.5 ± 0.7[a]	0.054 ± 0.008	1.6 ± 0.3

Values are means ± SEM for 26 rats.
[a] Differs significantly from mean value for frontal cortex.

concentrations of plasma perfusate. V_{max}, K_m, and K_D values for predicted influx were obtained from Table 1, whereas K_i values for competing NAAs were taken from Smith et al. (1987), assuming $K_i \cong K_m$. Predicted influx rates using this analysis were within 30% of measured values in each of the six brain regions. The differences were not statistically significant ($p > 0.05$).

DISCUSSION

This study demonstrates that the nonmetabolizable amino acid, ACHC, is transported across the blood–brain barrier by the cerebrovascular NAA system. The transport K_m for ACHC is one-fifth that of the more commonly used homologue, ACPC, and is comparable to values for several natural amino acids, such as L-methionine, L-isoleucine, and L-tyrosine. The results suggest that ACHC may be a useful tracer for in vivo studies of amino acid transport into brain.

Neutral amino acids are transported into brain by a common carrier that is located at the cerebral capillaries of the blood–brain barrier (Oldendorf, 1971; Hawkins et al., 1982). This transport system facilitates the brain uptake of 14 plasma NAAs and has greatest affinity for amino acids with large aliphatic or aromatic side chains, such as L-phenylalanine (Oldendorf and Szabo, 1976; Smith et al., 1987). Transport is sodium independent and inhibited competitively by BCH, the defining substrate for the L system of Christensen (Wade and Katzman, 1975; Momma et al., 1987). Saturable ACHC transport across the blood–brain barrier can be localized to the cerebrovascular NAA carrier, since both BCH and L-phenylalanine inhibit >90% of brain ACHC uptake. Significant contributions from other transport systems were not detected, as neither MeAIB nor L-lysine significantly reduced ACHC influx into brain.

Regional V_{max} values for cerebrovascular ACHC transport are comparable to values for other NAAs, such as L-phenylalanine and L-leucine (Smith et al., 1985; Momma et al., 1987), consistent with transport by the same carrier mechanism. The greater V_{max} in the frontal and parietal cortex than in the other brain regions is consistent with the greater vascularity and thus capillary surface area of the cortex (Hawkins et al., 1982). Similar regional differences were observed in both F and V_v (Smith et al., 1985).

There were no significant regional differences in K_m for saturable ACHC transport. The mean value for the six brain regions equaled 0.053 μmol/ml, which is greater than that for L-phenylalanine (0.011 μmol/ml), the plasma amino acid with greatest affinity for the transport system, but comparable to values for several other plasma amino acids, such as L-methionine (0.040 μmol/ml), L-isoleucine (0.056 μmol/

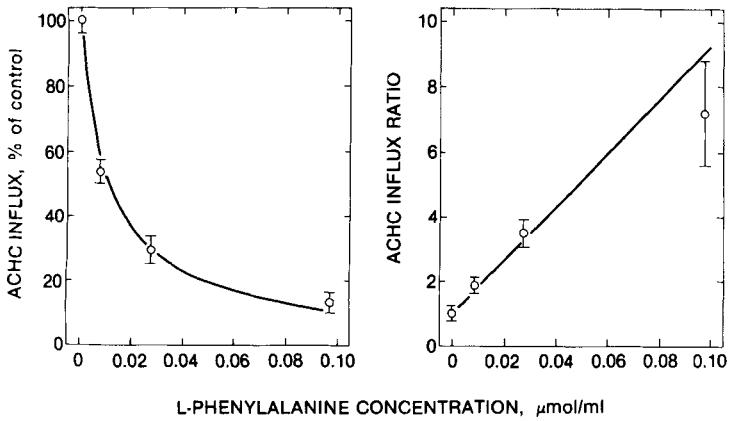

FIG. 2. Inhibition of ACHC influx into frontal cortex as a function of perfusate L-phenylalanine concentration. **Left:** ACHC influx versus L-phenylalanine concentration. **Right:** ACHC influx ratio $[(J_{in})_o/(J_{in})_i]$ versus L-phenylalanine concentration. Each point represents a mean for three animals. The lines illustrate the predicted influx and influx ratio, respectively, assuming K_i = 0.012 μmol/ml.

TABLE 2. *Effects of various amino acids on [^{14}C]ACHC influx into frontal cortex from saline perfusate*

Amino acid	Concentration (μmol/ml)	[^{14}C]ACHC influx (% of control)	K_i (μmol/ml)
BCH	1.0	9 ± 1[a]	0.099 ± 0.007
MeAIB	1.0	102 ± 7	—
L-Lysine	1.0	100 ± 6	—
ACHC	0.3	15 ± 2[a]	0.051 ± 0.005
ACPC	0.3	44 ± 4[a]	0.245 ± 0.042
ACBC	0.3	100 ± 8	—
	10.0	16 ± 2[a]	1.89 ± 0.25

Values are means ± SEM for three or four animals.
[a] Differs significantly from 100.

ml), and L-tyrosine (0.064 μmol/ml) (Smith et al., 1987). The value is approximately one-fifth that of ACPC, as determined from either self-saturation (0.29 μmol/ml; Smith and Takasato, 1986) or inhibition (0.25 μmol/ml) studies, and is approximately 1/35 that of ACBC. The similarity between K_m and K_i values for cerebrovascular NAA transport has been previously observed by Pardridge (1977) and Smith et al. (1987).

The differences in K_m between ACHC, ACPC, and ACBC are consistent with the known properties of the cerebrovascular NAA carrier. The transport K_m for an α-NAA at the blood–brain barrier is determined in large part by side chain hydrophobicity. For the 14 plasma NAAs with measurable affinity for the carrier, there is a linear relationship between $1/K_m$ and hydrophobicity as measured by the octanol/water partition coefficient (Smith et al., 1987). The three nonmetabolizable amino acids differ not only in ring size but in ring hydrophobicity. Each additional CH_2 group increases the partition coefficient by approximately threefold (Leo et al., 1971). Calculated K_m values from partition coefficients and the empirical relationship between the partition coefficient and $1/K_m$ predict a fivefold difference in K_m between ACHC and ACPC and a 20-fold difference between ACHC and ACBC. The similarity between the predicted and the measured differences suggests that the measured K_m differences can be explained primarily in terms of side chain hydrophobicity. Although further increase in ring size beyond that of ACHC would be expected to produce compounds with lower K_m, this may not occur because of steric limitations in the binding site (Washburn et al., 1981).

DeFeudis (1986) has suggested that published K_m values for cerebrovascular NAA transport as measured with the brain perfusion technique, as well as other methods, may be in error because influx values were not corrected for NAA metabolism in brain endothelial cells. If the rate of metabolism in the endothelial cell were significant compared to the rate of transport, then simple measurements of brain tracer uptake would overestimate NAA flux from plasma to brain extracellular fluid. To support this hypothesis he pointed out that the K_m for the nonmetabolizable NAA, ACPC, differed markedly from that for metabolizable NAAs, such as L-leucine and L-isoleucine. However, our results with ACHC would appear to indicate that endothelial cell metabolism does not lead to large errors in the measurement of transport K_m. Our value for the K_m of ACHC is comparable to that of L-leucine and L-isoleucine. The three NAAs have similar octanol/water partition coefficients (Leo et al., 1971). In addition, our value for the K_i of L-phenylalanine, as measured from the inhibition of [^{14}C]ACHC influx into brain, agrees well with the reported K_m for L-phenylalanine transport (Momma et al., 1987). The differences in K_m between ACPC and L-leucine and L-isoleucine result most likely from differences in side chain hydrophobicity (Leo et al., 1971; Smith et al., 1987) and not from endothelial metabolism artifacts.

ACHC influx from plasma is only ~9% of that from saline at the same concentration because of transport inhibition by competing amino acids. Cerebrovascular NAA transport is sensitive to competition effects because 14 plasma NAAs share the same transport system and because K_m values are low, comparable to plasma concentrations (Pardridge, 1977; Smith et al., 1987). As a result, the transport system is nearly (~96%) saturated with amino acids as a group, and each NAA must compete for available transport sites. Competition increases the K_m(app) 10 to 25-fold (Smith et al., 1985, 1987) and makes the saturable influx of each NAA dependent on the plasma concentrations of all competing amino acids.

In addition to ACHC transport by the cerebrovascular NAA carrier, there is a small nonsaturable com-

TABLE 3. *Regional ACHC influx into rat brain: comparison of predicted and measured values*

Brain region	ACHC influx (μmol/s/g × 10^6) Measured	Predicted	Percent difference
Frontal cortex	3.8 ± 0.4	3.3 ± 0.4	−13
Parietal cortex	3.8 ± 0.4	3.6 ± 0.3	−5
Occipital cortex	3.7 ± 0.5	2.6 ± 0.3	−30
Hippocampus	3.1 ± 0.3	2.3 ± 0.2	−26
Caudate nucleus	3.4 ± 0.4	2.7 ± 0.3	−21
Thalamus-hypothalamus	3.4 ± 0.4	2.5 ± 0.2	−26

Values are means ± SEM for five animals. Measured influx was obtained by brain perfusion with rat plasma containing 0.00289 ± 0.00015 μmol/ml ACHC. Predicted influx was calculated using Eqs. 3 and 5. Values of V_{max}, K_m, and K_D for ACHC were obtained from Table 1 and values for K_i for competing NAAs were taken from Smith et al. (1987). Amino acid concentrations of plasma perfusate equaled (in μmol/ml): Phe, 0.057 ± 0.006; Trp (free), 0.016 ± 0.003; Leu, 0.121 ± 0.014; Met, 0.053 ± 0.007; Ile, 0.064 ± 0.007; Tyr, 0.055 ± 0.006; His, 0.079 ± 0.009; Val, 0.114 ± 0.012; Thr, 0.215 ± 0.034; Gln, 0.458 ± 0.053; Asn, 0.036 ± 0.003; Ser, 0.179 ± 0.012; and Ala, 0.332 ± 0.037.

ponent of ACHC uptake into brain. The nonsaturable component may reflect passive diffusion of ACHC across the capillary cell membrane. Amino acids are known to cross lipid bilayers by passive diffusion (Klein et al., 1971) and the K_D for ACHC ($1.0–1.6 \times 10^{-4}$ ml/s/g) approximates the value predicted for passive diffusion across the blood–brain barrier (Rapoport et al., 1979; Yunger and Cramer, 1981; Takasato et al., 1984). Alternatively, the K_D may reflect the presence of a low-affinity (high K_m) transport system (Weissbach et al., 1982) or may arise as an artifact of measurement of J_{in} under nonsteady-state conditions (Cunningham et al., 1986) or from consistent underestimation of tracer in the brain vasculature. This last possibility is significant because q^*_{br} was not corrected for [^{14}C]ACHC in the brain endothelial compartment. Under most condition such a correction is not required because the volume of the brain capillary endothelium is very small (~0.001 ml/g, Gjedde and Christensen, 1984) and thus would be expected to contribute minimally to brain tracer content. However, at high perfusate ACHC concentrations (>1 μmol/ml), little [^{14}C]-ACHC enters brain and therefore tracer in the endothelial compartment may comprise a significant fraction of the total. For example, it can be estimated that failure to correct q^*_{br} for endothelial tracer will result in a 25% overestimate in J_{in} at a perfusate concentration of 10 μmol/ml ACHC. At lower concentrations (≤1 μmol/ml), the error would be <5%. These calculations assume an endothelial cell volume of 0.001 ml/g and equal concentrations of [^{14}C]ACHC in perfusate and endothelial cell (Gjedde and Christensen, 1984). Regardless of the cause or mechanism, the "nonsaturable" component contributes minimally (~7%) to influx at normal plasma NAA concentrations and therefore in most situations can be ignored. Its presence apparently does not lead to significant errors in calculated V_{max} or K_m, as evidenced by the close agreement between the values obtained using the entire data set (0–10 μmol/ml) and the values obtained using only the influx data at low ACHC concentrations (≤1 μmol/ml), where the contribution of the nonsaturable component is negligible.

Finally, the transport constants (V_{max}, K_m, K_D), in addition to providing information on the mechanisms of NAA transport into brain, allow prediction of in vivo influx rates for any given set of plasma NAA concentrations. Previous studies have demonstrated that for anesthetized rats predicted influx rates agree with measured rates within a factor of approximately two (Pardridge, 1983; Smith et al., 1987). However, in those studies, the predicted influx rates were not calculated using the exact plasma NAA concentrations from the animals that were used to determine influx experimentally. Since plasma NAA concentrations vary from animal to animal, some difference between measured and predicted rates might be expected. In this study, we demonstrate that agreement between predicted and measured influx rates is actually much better than twofold when plasma NAA concentrations are measured in the same animals as are used to determine influx. The predicted values came within 5–30% of measured values and the small differences were not statistically significant. The close agreement lends support to the transport model and suggests that the transport constants can be used to obtain accurate estimates of influx in healthy, pentobarbital-anesthetized rats. Since pentobarbital anesthesia produces only small changes in brain NAA uptake (Hawkins et al., 1982), it is likely that the kinetic constants can also be used to obtain reasonable estimates of influx in normal, awake rats.

In conclusion, [^{14}C]ACHC should prove useful as an in vivo probe of NAA transport between plasma and the CNS. It allows accurate in vivo measurements of influx without errors due to tracer metabolism or incorporation into proteins. In addition, it may allow determination of brain NAA concentration and NAA efflux from brain. [^{14}C]ACHC is well suited for use with quantitative autoradiography, which measures regional brain radioactivity but provides no information on the chemical form of the tracer. Furthermore, if labeled with a positron emitting radionuclide, ACHC could be used to examine amino acid transport in patients with diseases such as phenylketonuria (Washburn et al., 1982).

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