Effect of Aging on the Kinetics of Blood–Brain Barrier Uptake of Tryptophan in Rats

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Purpose. The purpose of this investigation was to examine the effect of aging on the blood-brain barrier (BBB) transport of tryptophan. ***Methods.*** A well established in-situ brain perfusion technique was used to examine brain uptake of ^{14}C-tryptophan in 2-, 12- and 24-month old Sprague-Dawley rats; perfusate tryptophan concentrations ranged from 0.00175 to 2 mM. Uptake data were modeled using non-linear regression analysis. ***Results.*** Permeability-surface area product (PA) for tryptophan was significantly lower in 12- and 24-month old rats, as compared to the 2-month old animals. A transport model consisting of both saturable (Michaelis-Menten type) and non-saturable components best described brain uptake of tryptophan in all 3 age groups. However, age-dependent differences in BBB transport parameters of tryptophan were observed. For the saturable component, both Vmax and Km were significantly lower in the 12- and 24-month old rats, as compared to the youngest group of rats. These results suggest that transporter mobility, number and affinity for tryptophan are altered in older rats. Values for K_d, the rate constant for non-saturable brain tryptophan transport, were also significantly lower in animals of the two older age groups. Interestingly, PA values for thiourea, a compound believed to be transported across BBB by diffusion, were also lower in these two age groups. ***Conclusions.*** Aging decreases the ability of the BBB to transport the neutral amino acid tryptophan.

KEY WORDS: blood–brain barrier: aging; tryptophan; transport; rats.

INTRODUCTION

About 10–15% of the elderly population (there is an estimated 30 million Americans over age 65) have mood alterations such as dementia and depression (1). One major cause of such central nervous system (CNS) dysfunction is altered neurotransmitter concentrations in the brain (2). Age-related decreases in brain serotonin, one of the important neurotransmitters responsible for modulating behavior, were found in both animals (rat and monkey) and humans (3–5). However, mechanisms responsible for this decrease have not been adequately evaluated. In principle, a complete metabolic picture of serotonin is required to understand this phenomenon. Age-related studies on the blood-brain barrier **(BBB)** transport of tryptophan (its amino acid precursor), formation and degradation kinetics of this neurotransmitter in the brain along with its receptor(s) affinities and efficiency of pre-synaptic re-uptake in aging should provide valuable insights.

As a first step in this direction, the objective of the present study was to examine the effect of aging on brain uptake of tryptophan. In young animals (19-day and 5 to 6-mo old rats), tryptophan is transported across the **BBB** by saturable (carrier) and non-saturable mechanisms (6,7). Little is known about this subject in old animals. In this connection, it is of interest to note that the **BBB** transport of choline and I-DOPA (precursors for acetylcholine and dopamine, respectively) are reduced with aging in non-human primates and rats (3,8,20). Since both choline and tryptophan cross the BBB by similar (i.e. carrier-mediated and simple diffusion) mechanisms (6,8), transport of tryptophan across this barrier may also be affected in aging. Therefore, in this study, the kinetics of this transport was investigated in rats of three age groups (young adult, mature adult and old). Our results showed that brain uptake of tryptophan was altered in aging. In older rats, tryptophan transporter capacity decreased (lower Vmax) with an increase in the affinity of the carrier (lower Km) for the amino acid. The non-saturable (most likely, passive diffusion) component (K_d) of this amino acid also decreased with aging.

MATERIALS AND METHODS

Radioactive Materials. ^{14}C-tryptophan (53.8 mCi/mmol), diazepam (53.8 mCi/mmol), thiourea (58.0 mCi/mmol) and sucrose (4.2 mCi/mmol) were purchased from New England Nuclear Corp., Boston, MA.

Animals. Sprague-Dawley rats, ages of 2-, 12- and 24-mo old, were obtained from Harlan (Indianapolis, IN) or National Center for Toxicology Research (Jefferson, AR). Animals were exposed to a 12-hour light:dark cycle and housed two per cage. Rats were fed with commercial rat chow and tap water ad libitum and were allowed an acclimatization period of at least 4 days prior to experimentation.

Surgical Procedure. The in-situ brain perfusion technique developed by Takasato et al (9) was used with minor modifications. Briefly, the right occipital and superior thyroid arteries were sealed by cauterization in the anesthetized (sodium pentobarbital, 50 mg/kg, i.p.) rat. Then, the nght pterygopalatine artery was ligated. A catheter (PE-50 tubing) filled with heparinized (30 IU/ml) normal saline was placed in the right external carotid artery. Finally, the right common carotid artery was ligated posterior to the bifurcation of external carotid artery followed by severing of the left heart ventricle (to eliminate potential flow contributions from the systemic circulation) immediately prior to initiation of perfusion. The perfusate consisted of pH 7.4 bicarbonate-buffered physiologic saline (128 mM NaCl, 24 mhf $NaHCO_3$, 4.2 mM KCI, 2.4 mM NaH_2PO_4, 1.5 mM $CaCl_2$, 0.9 mhf $MgCl_2$, and 9 mM glucose) containing various concentrations (0.00175, 0.01, 0.025, 0.05, 0.2, 0.5 and 2 mM) of tryptophan (^{14}C-labeled and unlabeled). Prior to use, the perfusate was filtered, oxygenated and heated to 37°C. Then, it was pumped via the external carotid artery into the right hemisphere of the brain at a rate of 10 ml/min (to minimize back-

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flux of amino acid transported into brain during perfusion) for 30 seconds, a time period over which transport was shown to be unidirectional (10) for tryptophan. Following perfusion, the animal was decapitated and 5 (frontal, parietal and occipital lobes, hippocampus and caudate nucleus) brain regions were collected from the right hemisphere and counted for radioactivity.

Calculations. Brain uptake of tryptophan was expressed in terms of two related parameters: cerebrovascular permeability-surface area product (PA) and unidirectional influx (Jin). The first parameter (PA) was determined from parenchymal brain uptake of ^{14}C-tryptophan as following:

$$PA = -F * \ln[1 - Q_{br}/(C_{pf} * T * F)] \quad (1)$$

where F is regional cerebral perfusion fluid flow determined by ^{14}C-diazepam (discussed later), T is the net perfusion time, Cpf is the perfusate concentration of tryptophan and Q_{br} is the parenchymal brain content of ^{14}C-tryptophan at time of sacrifice. This last parameter was calculated as follows:

$$Q_{br} = Q_{tot} - (Vv * C_{pf}) \quad (2)$$

where Q_{tot} is the measured brain content of ^{14}C-tryptophan and Vv is the brain intravascular volume determined by ^{14}C-sucrose (discussed later).

Permeability-surface area product (PA) values are related to Jin values by the equation:

$$Jin = F * (1 - e^{-PA/F}) * C_{pf} \quad (3)$$

Finally, various transport parameters (Vmax, Km and Kd) were obtained from fitting Jin data to a transport model (equation 4) involving both saturable (Michaelis-Menten) and non-saturable (first order) pathways:

$$Jin = (Vmax * Cpf)/[(Km + Cpf) + (Kd * Cpf)] \quad (4)$$

where Vmax is the maximal influx rate of the saturable transport component; Km is the half-saturation constant for tryptophan and Kd is the first order (non-saturable) transport rate constant.

Regional perfusion fluid flow (F), intravascular volume (Vv) and the BBB integrity were determined by using the above perfusion technique with appropriate ^{14}C-labeled tracers. Values for F were calculated from brain concentrations of ^{14}C-diazepam (its brain uptake is flow dependent) using the relationship:

$$F = Q_{br}/(T * C_{pf}) \quad (5)$$

Value for Vv (%) was estimated from brain tissue ^{14}C-sucrose (essentially an impermeable compound) as follows:

$$Vv = 100 * [(\text{dpmlg brain})/(\text{dpm/ml perfusate})] \quad (6)$$

Blood-brain barrier integrity was tested by calculation of PA for ^{14}C-thiourea (it is transported across BBB by diffusion) based on equation 1.

Data Analysis. The Jin data were analyzed using a regression method originally reported by Atkins and Nimmo (11) and used by others (12). Briefly, the 21 Jin observations (7 tryptophan concentrations, each in triplicate) for each brain region were randomly divided into three data sets (A. B and C) such that each set had one observation from each tryptophan concentration. Then, best-fit values for the transport constants (Vmas. Km and Kd) were obtained from a weighted non-linear regression analysis (13) of each data set. All parameters (F, Vv, PA. Jin. Vmax. Km and Kd) were tested for age-related differences by using analysis of variance (ANOVA) followed by Scheffe's multiple range test (14). Outliers were dropped using the Dixon's test for extreme values (15). All values are presented as mean ± SE (n = 3 – 5) and the criterion for statistical significance was $p < 0.05$.

RESULTS

Regional F and Vv values are shown in Table 1. These parameters were similar among the various age groups and regions. Permeability-surface area product (PA) of ^{14}C-thiourea is also shown in Table 1. No regional differences were observed within a given age group. However. these values were higher in the 2-mo old rats as compared to those in the 12-mo old rats, with differences in 4 (frontal. parietal

Table 1. Cerebral Perfusion Fluid Flow, Intravascular Volume and PA for Thiourea in Rats of Various Ages"

Regions	Perfusion Fluid Flow[b] (ml/s * g) * 10^2			Intravascular Volume[c]			PA of Thiourea (ml/s * g) * 10'		
	2-mo	12-mo	24-mo	2-mo	12-mo	24-mo	2-mo	12-mo	24-mo
Frontal lobe	7.8 (1.73)	9.3 (0.56)	8.8 (2.1)	0.82 (0.091)	0.72 (0.049)	0.67 (0.058)	9.25[a,b] (0.41)	6.40[a] (0.36)	6.11' (0.15)
Parietal lobe	8.5 (1.5)	11 (1.2)	10 (1.8)	0.66 (0.057)	0.68 (0.046)	0.67 (0.033)	8.38[c,d] (0.13)	5.77' (0.32)	6.16[d] (0.23)
Occipital lobe	8.1 (1.2)	10 (0.82)	8.9 (1.30)	0.72 (0.082)	0.59 (0.050)	0.54 (0.044)	8.89[e,f] (0.66)	5.27[e] (0.48)	5.81' (0.27)
Hippocampus	14 (1.5)	9.2 (0.35)	12 (0.54)	0.76 (0.11)	0.55 (0.015)	0.55 (0.062)	7.96 (0.54)	6.48 (0.57)	6.82 (1.45)
Caudate nucleus	7.7 (0.91)	5.0 (0.35)	4.7 (0.55)	0.40 (0.10)	0.53 (0.12)	0.38 (0.021)	8.91[g] (1.89)	5.0[g] (0.50)	5.39 (0.37)

[a] Numbers with same superscript indicate significant difference ($p < 0.05, n = 3 - 5$).
[b] Determined by ^{14}C-diazepam; values are mean (SE).
[c] Determined by ^{14}C-sucrose; values are mean (SE).

and occipital lobes and caudate nucleus) out of the 5 regions being statistically significant; PA values for thiourea in the 2-mo old rats were also higher than those in the 24-mo old rats; differences in the frontal, parietal and occipital lobes were statistically significant. Mean PA values for thiourea were essentially identical in the 12- and 24-mo old rats.

In all 3 age groups, PA values for tryptophan declined in a biphasic manner as its perfusate concentration was increased (see Fig. 1, the frontal lobe was chosen as a representative region). Permeability-surface area product (PA) values declined very rapidly in the concentration range 0.00175 to 0.05 mM; above that range (0.2 to 2 mM) PA values declined less rapidly. Profiles for the other 4 regions were similar to that observed with the frontal lobe. Details of PA values for various age groups and regions are presented in Table 2. Mean PA values for tryptophan decreased about 20-fold as its concentrations in the perfusate increased from 0.00175 to 2 mM for all regions in the 3 age groups. For concentrations up to 0.05 mM, mean PA values did not differ among the different age groups and regions (Fig. 1 and Table 2). At a perfusate tryptophan concentration of 0.2 mM, mean PA values were significantly higher in the 2-mo old rats than those in the 12-mo old rats for 4 (frontal and parietal lobes, hippocampus and caudate nucleus) out of the 5 regions. Mean PA values in the 2-mo old rats were also significantly higher than the corresponding values in the 24-mo old rats for all 5 regions. Though these values were lower in the 12-mo old group than those in the 24-mo old group, differences were not statistically significant (Table 2). At 0.5 mM, mean PA values were significantly higher for all 5 regions in the 2-mo old rats as compared to those in other 2 groups. with one exception (i.e. the hippocampus); in this region the PA value was not significantly different between the 2- and 24-mo old rats. In addition, mean PA values were lower in the 12-mo old rats as compared to those in the 24-mo old rats, with differences in 4 (parietal and occipital lobes, hippocampus and caudate nucleus) out of the 5 regions (Table 2) being statistically significant. At a perfusate concentration of 2 mM, mean PA values for tryptophan in the 2-mo old rats were significantly higher than those in other two groups, for all 5 regions. Between 12- and 24-mo old rats, mean PA values were higher in the younger rats; differences in the parietal lobe and caudate nucleus were statistically significant (Table 2).

The relationship between the unidirectional influx (Jin) for tryptophan and its concentration in the perfusate for the frontal lobe (representative data across regions and ages) in the 2-mo old rats is shown in Fig. 2; the individual contributions of the saturable and non-saturable components are also shown. Unidirectional influx (Jin) was found to be a saturable (curvilinear) function of the perfusate concentration up to 0.05 mM (inset, Fig. 2). Above that concentration, Jin increased linearly with the perfusate concentration. Profiles for influx (Jin) versus concentration for the 3 age groups are shown in Fig. 3. Transport constants (Vmax, Km, and Kd) for the various age groups and regions (calculated based on equation 4), are presented in Table 3. Mean Vmax values were higher in the 2-mo old rats than those in the 12-mo old rats, with differences in all 5 regions being statistically significant. Values for Vmax in the 2-mo old rats were also greater than those in the 24-mo old group. with differences in 3 (parietal and occipital lobes. and caudate nucleus) out of the 5 regions being statistically significant. Mean values for Vmax in the 12-mo old group were lower than those in the 24-mo old group; differences in the parietal and occipital lobes, hippocampus, and caudate nucleus were statistically significant.

Mean values for Km were significantly higher in the 2-mo old rats than those in the 12-mo old rats for all 5 regions. Mean Km values in the 12-mo old rats were lower than those in the 24-mo old rats: differences in the occipital lobe and caudate nucleus were statistically significant.

Mean values for the non-saturable transport rate constant. Kd. were significantly higher in the 2-mo old rats as compared to those in the 12-mo old rats for 4 (parietal and occipital lobes hippocampus and caudate nucleus) out of the

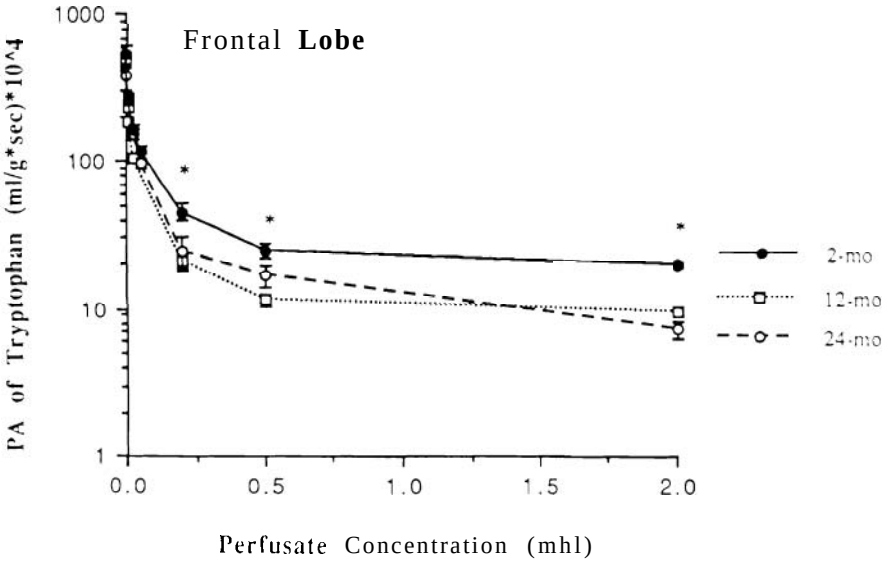

Fig. 1. Relationship between cerebrovascular PA and perfusate concentration of tryptophan in the frontal lobe for 2-, 12- and 24-mo old rats. 'denotes statistical significance, see text.

TABLE 2. Permeability-Surface Area Product Values for BBB Transport of Tryptophan in Rats of Various Ages[a]

	PA (ml/s * g) * 10^4						
Perfus. Conc. (mM)	0.00175	0.01	0.025	0.05	0.2	0.5	2
2-mo rats							
Frontal lobe	532 ± 40	247 ± 30	165 ± 24	119 ± 1.6	45 ± 6.5'	24 ± 1.2'	19 ± 1.6'
Parietal lobe	499 ± 47	251 ± 31	179 ± 24	125 ± 8.4	48 ± 6.5'	25 ± 1.0'	22 ± 0.25'
Occipital lobe	481 ± 19	230 ± 32	163 ± 29	118 ± 9.2	45 ± 12[d]	24 ± 0.32'	21 ± 1.5[b]
Hippocampus	381 ± 34	213 ± 13	154 ± 22	137 ± 31	45 ± 9.7'	26 ± 5.1	19 ± 2.0'
Caudate nucleus	422 ± 31	193 ± 37	163 ± 20	107 ± 12	41 ± 9.4'	19 ± 0.41'	18 ± 1.1'
12-mo rats							
Frontal lobe	467 ± 24	187 ± 0.71	107 ± 4.2	ND	21 ± 2.3	11 ± 0.58	9.4 ± 0.36
Parietal lobe	474 ± 34	194 ± 16	119 ± 17	ND	21 ± 1.7	12 ± 0.29[d]	9.4 ± 0.29'
Occipital lobe	442 ± 35	154 ± 3.2	96 ± 0.88	ND	21 ± 1.5	12 ± 0.88[d]	9.7 ± 0.078
Hippocampus	384 ± 62	144 ± 16	86 ± 2.6	ND	15 ± 1.8	8.4 ± 0.87'	8.8 ± 1.1
Caudate nucleus	393 ± 24	142 ± 16	76 ± 5.2	ND	1 8 1.6	9.9 ± 0.65[d]	8.1 ± 0.16'
24-mo rats							
Frontal lobe	375 ± 42	273 ± 7.0	152 ± 5.8	9 8 t 3.8	24 ± 3.4	17 21.6	7.2 ± 0.57
Parietal lobe	339 ± 44	260 ± 8.0	156 ± 6.2	90 ± 2.3	24 ± 1.9	17 ± 0.21	6.6 ± 0.30
Occipital lobe	377 ± 37	237 ± 6.0	142 ± 4.3	86 ± 2.9	26 ± 1.3	18 ± 0.70	7.3 ± 0.37
Hippocampus	346 ± 29	258 ± 15	138 ± 2.2	89 ± 1.3	28 ± 1.8	21 ± 1.8	6.1 ± 0.41
Caudate nucleus	320 ± 34	205 ± 5.0	113 ± 19	77 ± 4.0	21 ± 3.4	16 ± 1.2	5.3 ± 0.46

[a] Values are mean ± SE, n = 3; ND: not determined.
[b] Significantly higher than values in 12- and 24-mo old rats.
[c] Significantly different from values in 24-mo old rats.
[d] Significantly higher than values in 24-mo old rats.
[e] Significantly different from values in 2- and 24-mo old rats

5 regions. Values for the 2-mo old group were also greater than those in the 24-mo old group, with differences in 4 (frontal, parietal and occipital lobes and hippocampus) regions being statistically significant. In contrast to the data for Vmax and Km, mean values for Kd were higher in the 12-mo old rats than those in the 24-mo old rats, with differences in the parietal and occipital lobes being statistically significant.

DISCUSSION

The in-situ brain perfusion technique used in this study was chosen because it is a convenient and reliable method to determine BBB transport of amino acids (9). The three age groups used in this study represents young adult, mature adult and old rats, respectively (16). Values for F (determined using ^{14}C-diazepam) and Vv (determined using ^{14}C-sucrose) were similar across regions and age groups indicating that both perfusion fluid flow and regional blood volume are independent of age and region. On the other hand, PA values for thiourea (index of BBB integrity) were age dependent (discussed later). Values obtained for F, Vv and PA for thiourea in the 2-mo old rats in this study were in good agreement with those reported by the original investigators (9) in young rats and confirmed that the in-situ perfusion technique was accurately established in our laboratory. Though the pump infusion rate was maintained constant (10 ml/min) among the three groups, age-related changes in cerebral capillary density can alter the perfusion rate at the transport site. The essentially identical F values determined by ^{14}C-diazepam (its brain uptake is flow dependent) for the 3 age groups confirmed that perfusion rate at these site(s) were the same in all 3 age groups. Hence, differences in the brain uptake of thiourea and tryptophan (discussed later) observed in different animal groups reflect the effect of aging. Since the perfusion process can alter BBB, it is important to confirm that the perfusion rate used did not impair its integrity. Values for PA of thiourea and sucrose obtained in this study agreed with the values reported by the developers of this method (9) who compared their values with those measured after intravenous administration of the amino acid (17). The intravenous method does not cause disruption of the BBB.

Values of PA obtained for tryptophan in the 2-mo old rats were in good agreement with those reported by Smith et al (6). For example, they found that the PA value for the parietal lobe in 250–300 g (about 2 mo old) Osborne-Mendel rats was about 420 μmol/g * sec (estimated from Fig. 1 of their work); the corresponding value from the present study was 499 ± 47 μmol/g * sec. This good agreement provided additional confirmation about similarities in techniques used between the 2 laboratories. Permeability (PA) of tryptophan decreased about 20-fold as its perfusate concentration increased from 0.00175 to 2 mM in all 3 age groups. This profile is characteristic of canier-mediated transport systems. In addition, age-related decrease in the non-saturable transport (most likely, passive diffusion) of tryptophan would also tend to lower the estimates for tryptophan PA. In this connection, it is of interest to note that PA values for thiourea and Kd values for tryptophan were reduced by approximately the same extent (40–50%) (Tables 1 and 3) in the mature adult and old rats, as compared to the young animal. Thiourea is exclusively transported across the BBB by simple diffusion, while tryptophan is transported by both carrier-mediated (saturable) and passive diffusion (non-saturable) processes (Fig. 2). The similar magnitude of re-

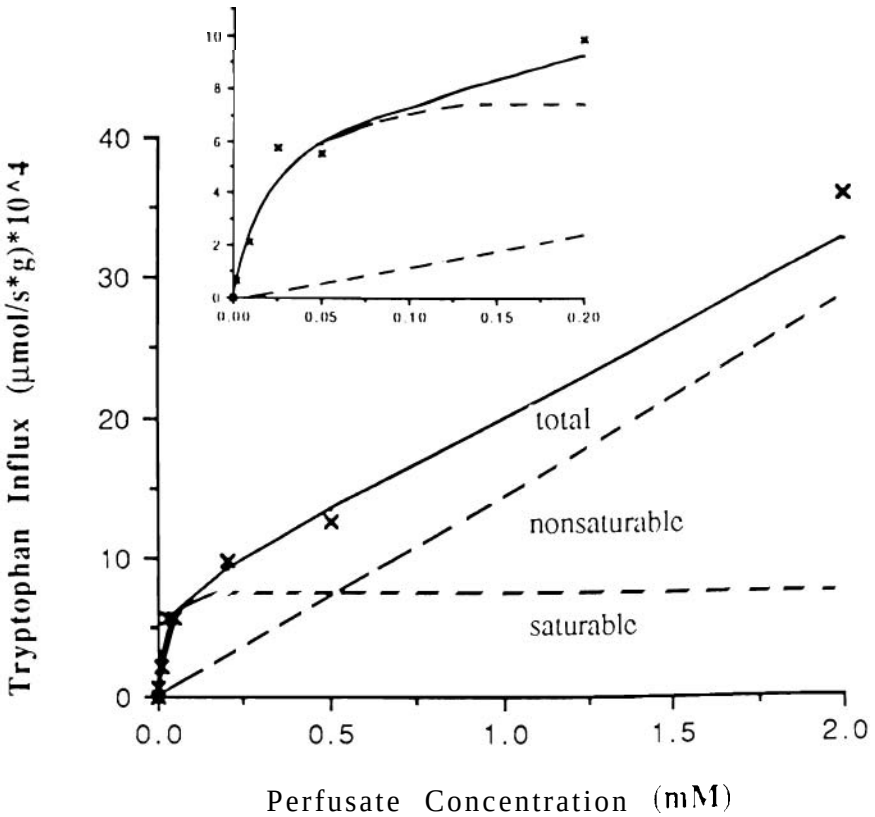

Fig. 2. Relationship between unidirectional influx (Jin) and perfusate concentration of tryptophan in the frontal lobe for the 2-mo old rats data set A, (Inset: Expanded scale. 0 to 0.2 mM).

duction in the PA for thiourea and the Kd component for tryptophan transport for the 12- and 24-mo old rats supports the hypothesis that the non-saturable component for tryptophan transport reflects a diffusion process. This reduction may be related to the increased the thickness of the basement membrane of the cerebral endothelium cells in older animals (2).

A model, which consists of a saturable and nonsaturable component (equation 4), was found to best describe Jin data for all 3 age groups, indicating that kinetics of brain uptake of tryptophan was qualitatively similar among the various ages. The observed differences (i.e. Vmax, Km and Kd) in the senescent animals show quantitative changes in BBB transport of tryptophan with aging. High (relative to 12- and 24-mo old rats) Vmax values observed in the 2-mo old rats reflect the higher requirement of amino acids in this age group to maintain high brain activities and protein synthesis in the brain of (developing) young rats. Values for Vmax and Km, especially the former (Table 3), were higher in the 24-mo old rats than those in the 12-mo old rats for all 5 regions; these results were unexpected and the reasons remain elusive.

Transport maximum (Vmax) is a function of both carrier mobility and carrier number, while Km is directly related to carrier mobility and carrier-substrate dissociation constant (8). Therefore, the observed lower Vmax and Km in the 12- and 24-mo old rats suggest an age-related reduction in the transporter capacity of tryptophan and enhanced affinity of the canier for the amino acid. It has been reported that two carrier systems (one with high-affinity, lou-capacity and the other with low-affinity, high-capacity) are involved in BBB transport of tryptophan (6). Values for Km in the 2-mo old rats obtained in this study were only about 1% of values found in other non-CNS tissues, such as liver, intestine and kidney (19). Therefore, our results suggest that the high-affinity, low capacity carrier plays a major role in tryptophan transport across the BBB as was speculated (18). In principle, the Kd component for tryptophan transport might also reflect a low-affinity, high-capacity carrier involved (7,18). However, due to similar age-related decreases in PA for thiourea and Kd for tryptophan (as discussed), this mechanism appears to be passive diffusion.

Values for Vmax and Km obtained in this study for the young rats were comparable with the reported values (6). Using the same perfusion technique as in this study, Vmax and Km in the parietal lobe of Osborne-Mendel rats (about 2-mo old) were 9.1 * 10^{-4} μmol/g * sec and 0.015 mM. respectively: the corresponding values from the present study were 7.1 * 10^{-4} μmol/g * sec and 0.020 mhi, respectively (Table 3). Using the brain uptake index (BUI) method. Sarna et al (7) showed that values for whole brain of Vmax and Km were 7.83 * 10^{-4} μmol/g * sec and 0.266 mM, respectively, in 19-day old Porton rats; the corresponding values in 5 to 6-mo old rats were 5.2 * 10^{-4} μmol/g * sec and 0.252 mhi, respectively. Values obtained for Vmax in the 2-mo old rats (Table 3) in this study were in good agreement with the

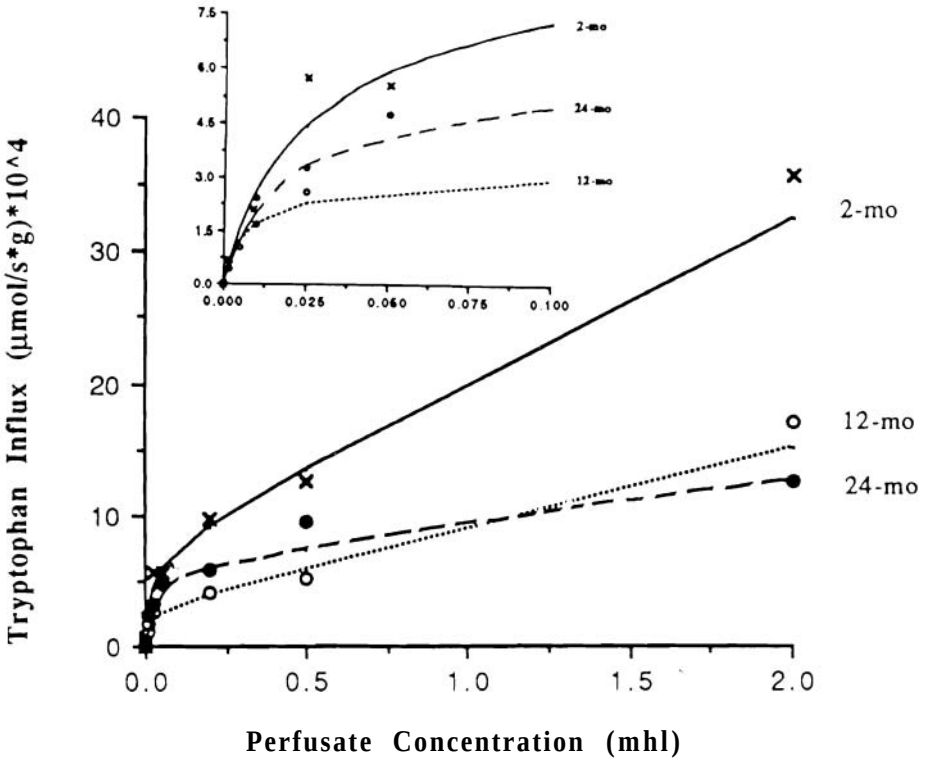

Fig. 3. Relationship between unidirectional influx (Jin) and perfusate concentration of tryptophan in the frontal lobe for the 2-, 12- and 24-mo old rats (data set A). (Inset: Expanded scale, 0 to 0.1 mM).

values reported by Sarna et al. (7). However, a 10-fold difference in Km values obtained with the BUI and in-situ brain perfusion techniques (this study and the study of Smith et al) *(6)* was observed. With BUI technique, there is some mixing of the injected solution with blood and neutral amino acids present in blood can competitively inhibit tryptophan transport. The latter process will lead to a higher Km value. This hypothesis was confirmed by Takasato et al (12); using the in-situ perfusion technique, they found that "apparent" Km exceeded the true Km by about 20-fold as the perfusate contained other neutral amino acids. Mean Kd values for tryptophan obtained in this study for the various regions in the 2-mo old rats are about 10-fold greater than those reported by Smith et al (6): this difference is most likely due to higher infusion rate (about 2-fold greater) used in the present study.

Rapoport et al (16). using sucrose as the tracer. reported that BBB permeability did not alter with age in rats. In the present study we also did not find any age-related changes in brain uptake of sucrose (indicated by Vv). But PA values for thiourea (BBB integrity marker) were significantly decreased in senescent rats. This finding suggests that sucrose is not a suitable marker to estimate BBB permeability, because it is a highly impermeable substance (i.e. negligible passive diffusion). Age-related changes in its brain uptake are more likely to be detected when BBB integrity is impaired (i.e. paracellular transport due to opening of tight

Table 3. Kinetic Constants for BBB Transport of Tryptophan in Rats of Various Ages"

	Vmax (µmol/s * g) * 10^4			Km (mM)			Kd (ml/s * g) * 10^4		
REGIONS	2-mo	12-mo	24-mo	2-mo	12-mo	24-mo	2 mo	12-mo	24-mo
Frontal lobe	7.4"	2.6"	5.8	0.023^{a}	0.0059^{a}	0.014	11^{a}	7.7	4.2^{a}
	(0.81)	(0.17)	(0.52)	(0.0046)	(0.00080)	(0.032)	(1.44)	(0.70)	(0.53)
Parietal lobe	$7.1^{b,c}$	$2.9^{b,d}$	$4.9^{c,d}$	0.020^{b}	0.0062^{b}	0.016	$13^{b,c}$	$8.1^{b,d}$	$3.7^{c,d}$
	(0.35)	(0.50)	(0.49)	(0.0017)	(0.0016)	(0.0037)	(1.14)	(0.67)	(0.64)
Occipital lobe	$6.9^{e,f}$	$2.2^{e,g}$	$4.8^{f,g}$	0.019^{c}	$0.0050^{c,d}$	0.014^{d}	$13^{e,f}$	$8.6^{e,g}$	$5.4^{f,g}$
	(0.56)	(0.14)	(0.16)	(0.0015)	(0.00030)	(0.0015)	(0.91)	(0.65)	(0.12)
Hippocampus	6.7^{h}	$2.1^{h,i}$	5.4^{i}	0.021^{e}	0.0056^{e}	0.016	$12^{h,i}$	4.7^{h}	4.3^{i}
	(0.34)	(0.17)	(0.37)	(0.0032)	(0.0017)	(0.0020)	(1.53)	(0.75)	(1.04)
Caudate nucleus	$6.6^{j,k}$	$1.9^{j,l}$	$4.0^{k,l}$	0.025^{f}	$0.0053^{f,g}$	0.016^{g}	9.2^{j}	7.3^{j}	3.8
	(0.46)	(0.26)	(0.30)	(0.0023)	(0.00070)	(0.0032)	(1.23)	(0.95)	(0.31)

[a] Values are mean (SE), $n = 3$; numbers with same superscript indicate significant difference ($p < 0.05$).

junctions) as compared to alterations in barrier permeability (i.e. transcellular transport across the bamer).

This is the first report on the effect of aging on the BBB transport of amino acids. Aging related lower brain uptake of tryptophan can, in principle, lead to reduced brain serotonin concentrations in the elderly, which consequently can result in mental dysfunction in this age group. Such confirmatory studies will be the topic of future investigations.

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REFERENCES

1. D. Cohen and C. Eisdorfer. Depression. In The Practice of Geriatrics, edited by E. Calkins, P. J. Davis and A. B. Ford, pp 185–205, W.B. Saunders Company, Philadelphia, 1986.
2. A. D. Mooradian. Effect of aging on the BBB. Neurobiol. Aging 9:31–39, 1988.
3. J. M. Ordy and K. R. Brizzee. Neurobiology and aging in nonhuman primates. In Neurobiology of Aging, edited by J. M. Ordy and K. R. Brizzee, Parts II, IV & VIII, pp 104. 260–261 and 575–593, New York Plenum, 1975.
4. L. D. Lytle and A. Altar. Diet, central nervous system and aging. Fed. Proc. 38:2017–2022, 1979.
5. K. R. Brizzee. Quantitative histological studies on aging changes in cerebral cortex of Rhesus monkey and Albino rat with notes on effects of prolonged low-dose ionizing irradiation in the rat. Prog. Brain Res. 40:141–160, 1973.
6. Q. R. Smith, S. Momma, M. Aoyagi, and S. I. Rapoport. Kinetics of neutral amino acid transport across the BBB. J. Neurochem. 49:1651–1658, 1987.
7. G. S. Sarna, M. D. Tricklebank, B. D. Kantamaneni, A. Hunt, A. Patel, and G. Cuzon. Effect of age on variables influencing the supply of tryptophan to the brain. J. Neurochem. 39:1283–1290, 1982.
8. A. D. Mooradian. Blood-brain barrier transport of choline is reduced in the aged rat. Brain Res. 440:328–333, 1988.
9. Y. Takasato, S. I. Rapoport, and Q. R. Smith. An in situ brain perfusion technique to study Cerebrovascular transport in the rat. Am. J. Physiol. 247:H484–493, 1984.
10. Y. Takada, D. T. Visstica, N. H. Greig, D. Purdon, S. I. Rapoport, and Q. R. Smith. Rapid high-affinity transport of a chemotherapeutic amino acid across the BBB. Cancer Res. 52:2191–96, 1992.
11. G. L. Atkins and I. A. Nimmo. Current trends in the estimation of Michaelis-Menten parameters. Anal. Biochem. 104:1–9, 1980.
12. Y. Takasato, S. Momma, and Q. R. Smith. Kinetic analysis of cerebrovascular isoleucine transport from saline and plasma. J. Neurochem 45:1013–1020, 1985.
13. SCI Software Statistical Consultants Inc., PCNONLIN User's Guide, edited by Statistical Consultants Inc., Lexington, KY, 1989.
14. SPSS User's Guide, edited by SPSS Inc., McGraw-Hill, New York, 1983.
15. S. Bolton. Transformation and outliers. In Pharmaceutical Statistics — Practical and Clinical Applications, edited by J. Swarbrick, pp 294–299, Marcel Dekker Inc., New York and Basel, 1984.
16. S. I. Rapoport. K. Ohno and K. D. Pettigreu. Blood-brain barrier permeability in senescent rats. J. Gerontol. 34:162–169, 1979.
17. S. I. Rapoport, K. Ohno, and K. D. Pettigreu. Drug entry into brain. Brain Res. 172:354–359, 1979.
18. S. Momma, M. Aoyagi. S. I. Rapoport. and Q. R. Smith. Phenylalanine transport across the BBB as studied uith the in situ brain perfusion technique. J. Neurochem. 48:1291–1300, 1987.
19. W. M. Pardridge. Brain metabolism: a perspective from the BBB. Physiol. Rev. 63:1481–1535. 1983.
20. J. L. Meek, L. Bertilsson. D. L. Cheney. G. Zsilla. and E. Costa. Aging-induced changes in acetylcholine and serotonin content of discrete brain nuclei. J. Gerontol. 32:129–132. 1977.