0013-7227/79/1053-0605$02.00/0
Endocrinology

Vol. 105, No. 3
Printed in U.S.A.

Carrier-Mediated Transport of Thyroid Hormones through the Rat Blood-Brain Barrier: Primary Role of Albumin-Bound Hormone*

WILLIAM M. PARDRIDGE†

Department of Medicine, Division of Endocrinology and Metabolism, UCLA School of Medicine, Los Angeles, Ca. 90024

ABSTRACT. The transport of [^{125}I]T_3 and [^{125}I]T_4 through the brain capillary wall, *i.e.* the blood-brain barrier, was studied in barbiturate-anesthetized rats using a tissue-sampling-carotid injection technique. The percent extraction of unidirectional influx of thyroid hormone during a single pass through the brain was measured relative to a highly diffusible [^{3}H]water reference. The K_m of T_3 transport was 1.1 μM; T_3 transport was inhibited by T_4 (K_i = 2.6 μM), rT_3 (K_i = 5.4 μM), and D-T_3 but not by 1000 μM concentrations of tyrosine, leucine, or potassium iodide. Bovine albumin also inhibited blood-brain barrier transport of T_3. The fractional inhibition of T_3 transport by albumin was a measure of the binding of T_3 by albumin *in vivo*, *i.e.* in the presence of a competing binding system, the BBB T_3 carrier. The apparent dissociation constant (K_d) of albumin binding of T_3 at the brain capillary level (76 μM) was 16-fold greater than the K_d of albumin binding of T_3 *in vitro* (4.7 μM), as determined by equilibrium dialysis. A model was derived that allowed for the *in vivo* application of the principles of the competitive ligand-binding assay; given apparent $K_d = K_d (1 + C/K_m)$, the local capillary T_3-binding capacity (C) may be calculated from the known values for apparent K_d, K_d, and K_m. Based on the relative binding index (C/K_m) of BBB binding of T_3 *vs.* the binding index of physiological concentrations of albumin, it may be estimated that about 10% of albumin-bound T_3 (which is 10-fold the fraction of dialyzable T_3) is transported into the brain on a single pass in the rat. (*Endocrinology* **105:** 605, 1979)

THE BRAIN is an important target organ for the action of the major thyroid hormones, T_3 and T_4. The thyroid hormones act on the brain to 1) increase the rate of glucose transport into the developing brain (1), 2) increase the rate of amino acid transport into brain (2) and amino acid incorporation into brain proteins (2, 3), and 3) influence the rate of monoamine synthesis in brain (4). Before the action of blood-borne thyroid hormones in brain, these substances must first be transported into the central nervous system through one of two barrier systems, either the brain capillary wall, *i.e.* the blood-brain barrier (BBB), or the choroid plexus, *i.e.* the blood-cerebrospinal fluid (CSF) barrier. Since the surface area of the BBB is at least 5000-fold that of the choroid plexus (5), it is likely that the major route of thyroid hormone transport into brain is via the BBB. Despite the importance of BBB transport of T_4 or T_3, there are few direct studies of thyroid hormone transport through this membrane. Ford and Gross (6) showed that the brain took up both T_4 and T_3 after an iv injection and that the uptake of T_3 was about 3-fold greater than that of T_4. Hagen and Solberg (7) reported quantitative data on the rate at which infused thyroid hormone entered the CSF and showed that the process mediating CSF uptake of T_4 was saturable, with an ED_{50} of approximately 15 μM total plasma T_4. Although it is likely that the locus of saturation of T_4 transport into CSF was at the BBB, measurements of the rate of T_4 or T_3 entry into CSF are not direct studies of BBB permeability. Transport into CSF is a function of the rate of compound influx into CSF via transport through the BBB or choroid plexus minus the rate of efflux of the compound from the CSF compartment, *e.g.* into brain cells or back to blood. Therefore, the present investigations were undertaken to determine whether the saturable transport system observed by Hagen and Solberg (7) for thyroid hormones exists at the BBB. Moreover, since the thyroid hormones are primarily bound to albumin in the rat (8), the effect of albumin binding on BBB transport of thyroid hormones was investigated. The presence of the putative BBB transport system for the thyroid hormones would provide a setting in which competition for ligand binding at the capillary level may occur between albumin and the transport system.

Received January 24, 1979.

* This work was supported by a grant from the NSF (BNS 78-05500) and a NIH Clinical Investigator Award (AM-00409). Presented in part at the Annual Meeting of the American Thyroid Association, Portland, OR, September 1978, and the Society for Neuroscience, St. Louis, MO. November 1978.

† To whom all correspondence and requests for reprints should be addressed.

Materials and Methods

Isotopes and reagents

Radiolabeled L-T_4 ([^{125}I]T_4; 0.8 μCi/pM), sodium iodide ([^{125}I]NaI; 2.1 μCi/pM), bovine albumin ([^{125}I]bovine albumin;

20 μCi/pM), and water ([^{3}H]water) were purchased from New England Nuclear Corp. (Boston, MA). Radiolabeled L-T_3 ([^{125}I]T_3; 2 μCi/pM) was purchased from Amersham (Chicago, IL). Radiochemical purity of the ^{125}I-labeled thyronines was at least 90% and was assassed by thin layer chromatography (Silical gel H, Analtech, Newark, DE) with the solvent system butanol-50-acetone-25-NH_4OH-18, followed by radioscanning (Berthold scanner).

Unlabeled bovine albumin (fraction V, nondefatted), L-T_4 L-T_3, sodium iodide, D-T_3, L-tyrosine, and L-leucine, were all purchased from Sigma Chemical Co. (St. Louis, MO); rT_3 was kindly provided by Dr. Inder J. Chopra.

Single injection studies

The transport of labeled T_4 or T_3 through the BBB was measured with a tissue-sampling-single injection technique (9) in barbiturate-anesthetized (45 mg sodium pentobarbital/kg ip) male Sprague-Dawley rats, weighing 250–350 g. A carotid artery (usually on the right side) was exposed and cannulated with a 27-gauge needle and an approximately 200-μl bolus (the exact volume is immaterial) of buffered Ringer's solution (5 mM Hepes, pH 7.4) was rapidly injected as a bolus. The injection solution contained 1.25–2.5 μCi/ml ^{125}I-labeled compound, 12.5–25 μCi/ml [^{3}H]water (a highly diffusible internal reference), and 0.025 g/dl bovine albumin (added to prevent the binding of labeled compounds to syringe walls or glass vials). At 15 sec after injection, a period sufficient for a single pass of the bolus through the brain but short enough to prevent recirculation, the rat was decapitated. Due to the bolus injection (9), there was no mixing of the labeled hormone with the circulating plasma proteins, so that only plasma proteins added to the injection solution bound the hormone at the capillary transport sites. After removal of the brain from the cranium, the cerebral hemisphere ipsilateral to the injection and rostral to the midbrain and an aliquot of the injection solution were solubilized in 1.5 ml NCS (Amersham) by shaking at 50 C for 2 h. The ^{3}H and ^{125}I radioactivity was counted simultaneously in a double isotope liquid scintillation counting system and after quench correction (see below), the brain uptake index (BUI) was calculated (9):

$$\text{BUI} = \frac{(^{125}\text{I dpm}) \div (^{3}\text{H dpm}) \text{ brain}}{(^{125}\text{I dpm}) \div (^{3}\text{H dpm}) \text{ injection solution}} \times 100$$

The BUI = E_T/E_R (10), where E_T and E_R are the percent extraction due to unidirectional influx of the ^{125}I test and [^{3}H]-water reference compounds, respectively, on a single pass through the brain. Since E_R = 75% (10) under the experimental conditions, the BUI overestimates E_T by about 25%.

Liquid scintillation ^{125}I quench curves

An essential feature of the BUI calculation is that it is a ratio of ratios, so that weights of brain or injection solutions cancel out (9). This obviates weighing samples and greatly improves the reproducibility of the technique. To preserve this feature, it was necessary to count the ^{125}I and ^{3}H radioactivity simultaneously, which was conveniently done in a liquid scintillation system using a Parkard Tri-Carb spectrometer (model 3320, Downers Grove, IL). Standard quench curves for ^{3}H were obtained using a [^{3}H]toluene standard. Quench curves for ^{125}I were obtained by adding a known radioactivity (~100,000 dpm) of [^{125}I]bovine albumin or [^{125}I]iodide to 10 scintillation vials containing 1 ml NCS. Ten milliliters of scintillation fluor (Liquifluor, Packard) were added to each vial along with 50–500 μl chloroform as a quenching agent. The radioactivity of the [^{125}I]albumin stock solution was standardized by counting in a Baird Atomic γ-spectrometer (Bedford, MA).

Competition studies

After assessing the BUI for [^{125}I]T_3 in Ringer's solution, various concentrations of unlabeled L-T_3, D-T_3, rT_3, L-T_4, L-leucine, L-tyrosine, potassium iodide, or bovine albumin were added to the injection solution, and the BUI for [^{125}I]T_3 was subsequently determined.

Efflux studies

The rate of efflux of [^{125}I]T_3 from brain subsequent to influx was studied by determining the BUI at 1, 2, or 4 min after carotid injection. Multiplying the BUI at each time point by the known concentration (10) of the water reference (E_R) yields the amount of ^{125}I remaining in the brain (E_T) at the respective time points. Analysis of these data by a log plot gives the rate constant (K) of ^{125}I efflux from the brain back to the blood. The K value relates to BBB permeability on the brain side of BBB, which is represented by the E_{eff}, *i.e.* the percent of brain T3 extracted by blood (11); $K = (E_{eff}F/V)$, where F is cerebral blood flow (0.6 ml $min^{-1}g^{-1}$), *i.e.* the whole brain value in the barbiturate-anesthetized rat (10), and V (in milliliters per g) is the ratio of the volumes of distribution of T_3 in the brain (in milliliters per g) to that in the blood (in milliliters per ml).

The symmetry of T_3 transport across the BBB may be examined by comparing the extraction of efflux (E_{eff}) to the extraction of influx (E_{inf}), as determined from the BUI (see above). A symmetrical transport system, *i.e.* one that mediates the bidirectional equilibrative (not concentrative) transport of substrate through the BBB, is characterized by comparable E_{inf} and E_{eff} (10). A transport system that actively transports thyroid hormone from blood into brain against a concentration gradient is characterized by $E_{inf} \gg E_{eff}$.

Equilibrium dialysis

The K_d of [^{125}I]T_3 binding to bovine albumin was determined from the law of mass action, K_d = (percent free ÷ percent bound) (albumin concentration). One and a half milliliters of the injection solution buffer containing 0.3 g/dl (44 μM based on a mol wt of 68,000) bovine albumin, 2 nM [^{125}I]T_3, and 0.05 g/dl sodium azide were added to a dialysis bag made of a 1.5 × 20-cm strip of dialysis tubing and dialyzed against 15 ml injection solution buffer at 37 C for 3.5 h; the dialysis bag was then rinsed and placed in 15 ml new buffer and dialyzed at 37 C for 20 h. The first dialysis served to remove any radiolabeled contaminants, *e.g.* [^{125}I]iodide, from the [^{125}I]T_3. The free to bound ratio was determined from the ratio of disintegrations per min/ml in the dialysis buffer to that in the dialysis bag.

Results

Liquid scintillation counting of ^{125}I

The liquid scintillation quench curves for ^{125}I in conventional ^{14}C and ^{3}H channels are shown in Fig. 1. These quench curves reflect the multiple nonγ (*e.g.* internal conversion electron) emissions of the ^{125}I isotope within the energy range of the ^{3}H and ^{14}C β-emissions. The quench of all experimental samples was greater than 0.4, *i.e.* greater than the point where there was a break in linearity of the ^{125}I quench curve in the ^{3}H channel (Fig. 1). To minimize the correction for ^{125}I spillover into the ^{3}H channel, a 10:1 ^{3}H:^{125}I radioactivity ratio was used in all injection solutions.

Inhibition of T_3 transport by analogs

The BUIs for T_3 and T_4 were 31.2 ± 1.5% and 15.8 ± 1.0%, respectively (mean ± SE; n = 4–6 rats). As shown in Fig. 2, the transport of [^{125}I]T_3 was saturable; the K_m (half-saturation constant) of T_3 transport was 1.1 μM. The possibility of a high affinity component of [^{125}I]T_3 transport was examined by adding a low dose (200 nM), of unlabeled T_3 to the injection solution. The BUI for T_3 was 30.9 ± 3.0 (mean ± SE; n = 3 rats), which was not significantly different from the control BUI, indicating the absence of a high affinity T_3 transport system. The transport of [^{125}I]T_3 was also inhibited by unlabeled T_4 with a K_i (half-inhibition constant) of 2.6 μM (Fig. 3). Therefore, the affinity of the transport system is 2.5-fold greater for T_3 than for T_4, which correlates with the 2-fold greater BUI for T_3 relative to T_4. In addition, the inhibition of labeled T_3 transport by unlabeled rT_3 was assessed. The BUI for [^{125}I]T_3 in the presence of 1, 5, and 20 μM rT_3 was 24.7 ± 5.5, 16.6 ± 2.5, and 10.7 ± 1.0, respectively (mean ± SE; n = 3 rats for each point); a double reciprocal plot of these data similar to Figs. 2 and 3 was linear (r = 0.99) and indicated that the rT_3 K_i was 5.4 μM.

The cross-inhibition of [^{125}I]T_3 transport by unlabeled analog was also observed for 20 μM D-T_3 but not for

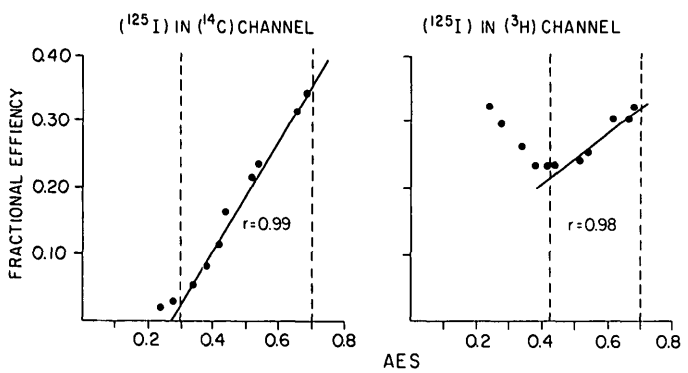

FIG. 1. Liquid scintillation ^{125}I quench curves at conventional settings for ^{3}H and ^{14}C β-emissions are obtained by plotting the fractional efficiency *vs.* the automated external standard (AES). The brackets define the linear portion of the quench curves. Corrections for ^{125}I spillover into the ^{3}H channel was minimized by using a 10:1 ^{3}H:^{125}I ratio in all studies involving simultaneous ^{3}H and ^{125}I counting.

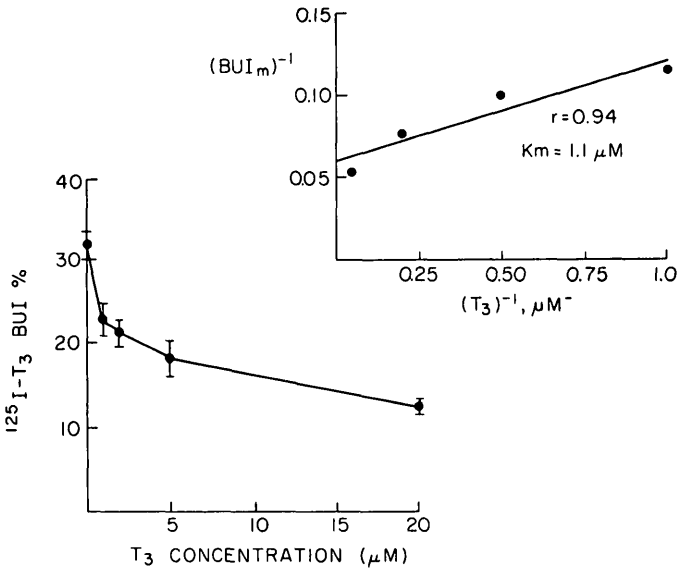

FIG. 2. The BUI for [^{125}I]T_3 is plotted *vs.* the concentration of unlabeled T_3 in the carotid injection solutions. Data are the mean ± SE (n = 3–4 rats). A double reciprocal linear transformation of the saturation data is obtained by plotting the reciprocal of BUI_m (BUI_m = BUI_0 − BUI, where BUI_0 is the uptake at a tracer concentration of T_3 and BUI is the uptake at the respective level of unlabeled T_3) *vs.* the reciprocal of the T_3 concentration. The K_m is obtained from the slope to intercept ratio. Since BUI_m is dimensionless, V_{max} cannot be obtained from the intercept. Details of the kinetic analysis have been reported previously (27).

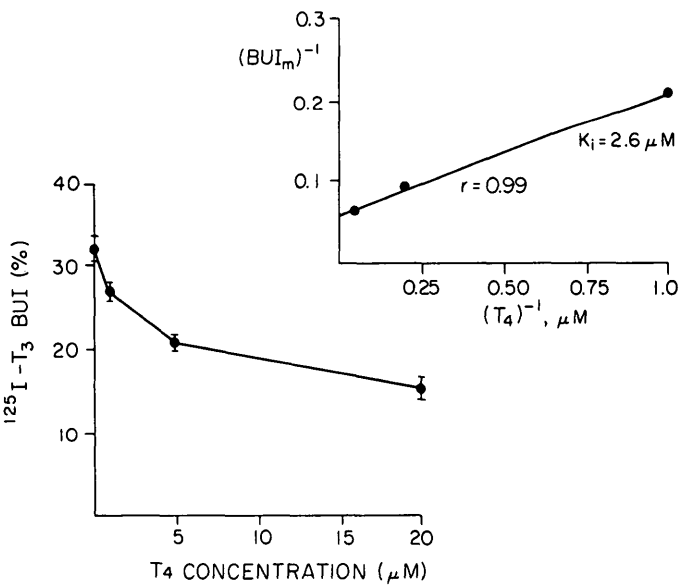

FIG. 3. The BUI for [^{125}I]T_3 is plotted *vs.* the concentration of unlabeled T_4 in the injection solution. Data are the mean ± SE (n = 3–4 rats). See Fig. 2 for details of the double reciprocal plot.

unlabeled tyrosine, leucine, or potassium iodide at 1000 μM concentrations (Table 1). These data indicate that the thyroid hormone transport system is 1) only weakly stereospecific and 2) not part of the previously described BBB transport system which mediates the flux of the neutral amino acids, *e.g.* tyrosine or leucine (12).

Inhibition of T_3 transport by albumin

Albumin is known to bind the thyroid hormones tightly (13). Since albumin is not transported through the BBB (7), the addition of bovine albumin to the injection solution would be expected to bind the hormone and inhibit BBB transport of T_3. As shown in Fig. 4, albumin inhibits T_3 transport; the fractional inhibition of T_3 transport by albumin (Fig. 4) is a measure of albumin binding of T_3 at the capillary level. Therefore, the slope of the double reciprocal plot (Fig. 4) gives the apparent (app) K_d (76 μM) of albumin binding of T_3 *in vivo*. The app K_d observed in these studies is more than 15-fold greater than the concentration of albumin (4 μM) which has been previously reported to cause 50% binding *in vitro* (13). Therefore, the K_d of T_3 binding to the preparation of albumin used in these studies was determined by equilibrium dialysis. The measured K_d (4.7 ± 0.1 μM) approximates previous estimates in the literature (13).

Since albumin is the major plasma protein which binds T_3 *in vivo* in the rat (8), the effect of rat serum on BBB transport of T_3 was investigated (Table 2). The BUI of T_3 in the presence of 67% rat serum approximated the value obtained at 3 g/dl albumin (Fig. 4). To determine the fraction of the BUI for T_3 in the presence of 67% rat serum that represented specific transport, as opposed to nonspecific adsorption to the capillary wall, a measure of the background BUI for ^{125}I-labeled compounds was needed. Since iodide does not cross the BBB (14), the BUI for [^{125}I]NaI was measured (Table 2). Assuming that the iodide BUI also represents nonspecific T_3 uptake, the fraction of specific T_3 transport in the presence of 67% rat serum may be calculated; given the BUI for T_3 in the absence (31.2%) or presence (8.8%) of 67% rat serum and using the iodide BUI (4.9%) as background, the fraction of specific T_3 transport is (8.8 − 4.9) ÷ (31.2 − 4.9) = 15%. This value is several-fold greater than the dialyzable fraction of [^{125}I]T_3; in the presence of 90% rat serum, the dialyzable fraction was 1.6 ± 0.2% (mean ± SE; n = 3), as determined by equilibrium dialysis. The effect of 67% human serum on [^{125}I]T_3 transport was also investigated (Table 2); human serum inhibits T_3 transport to a greater extent than did rat serum, consistent with binding of T_3

TABLE 1. Cross-inhibition of BBB [^{125}I]T_3 transport by unlabeled analogs

Analog[a]	BUI (%)
Control (0.1% albumin)	27.5 ± 1.5
20 μM D-T_3	18.7 ± 1.1
1000 μM L-Tyrosine	27.8 ± 2.4
1000 μM L-Leucine	29.0 ± 1.2
1000 μM Potassium iodide	30.2 ± 2.7

Values given are the mean ± SEM (n = 3-4).
[a] Bovine albumin (0.1%) was added to all injection solutions.

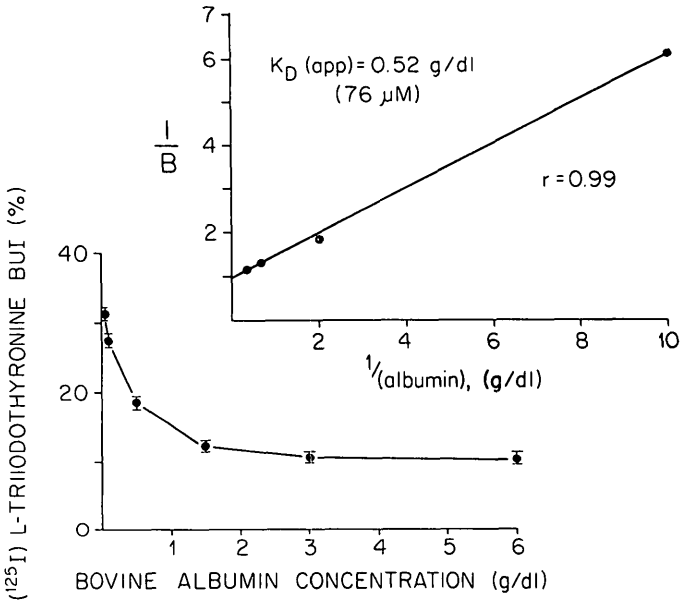

FIG. 4. The BUI for [^{125}I]T_3 is plotted *vs.* the concentration of bovine albumin (0.03-6 g/dl) in the injection solution. Data are the mean ± SE (n = 3-4 rats). The BUI for T_3 in the presence of 0.001 and 0.01 g/dl was unchanged (31.6 ± 1.4 and 31.9 ± 0.9, respectively) from the value at 0.03 g/dl. Since albumin inhibits T_3 transport by binding the hormone, the fractional inhibition of the BUI by the respective albumin concentration is a measure of the fraction (B) of T_3 bound to albumin at the capillary level, *i.e.* B is the fraction of T_3 bound to albumin in the presence of the T_3 transport system which is competing with albumin for T_3 binding. The calculation of B is as follows, B = (BUI_0 − BUI) ÷ (BUI_0 − BUI_{NS}), where BUI_0 is the uptake at the lowest dose of albumin (0.03 g/dl) where no inhibition is observed, BUI is the uptake at the respective albumin dose at which inhibition is observed, and BUI_{NS} is the nonspecific uptake (5%) represented by the BUI for an infinitely high concentration of albumin. The BUI_{NS} may be obtained by plotting $^1/(BUI_0 - BUI)$ *vs.* ^{1}/(albumin); such a plot (not shown) is linear (r = 0.99) and the y intercept of this linear transformation = BUI_{NS} = 5%. Since BUI_{NS} also equals the BUI for [^{125}I]iodide, the latter is assumed to represent the nonspecific uptake (see text). The slope of the ^{1}/B *vs.* ^{1}/albumin plot gives the app K_d of albumin binding of T_3 *in vivo* (see *Appendix*).

TABLE 2. Effects of rat and human serum on BBB transport of [^{125}I]T_3

^{125}I-Labeled compound	Injection solution	BUI (%)[a]
T_3	67% Rat serum	8.8 ± 0.2
T_3	67% Human serum	6.9 ± 0.1
Iodide	Ringer's (0.1 g/dl albumin)	4.9 ± 0.2

[a] Values given are the mean ± SE (n = 3-4).

by both albumin and thyroid-binding globulin in human serum(13).

Regional T_3 transport

The hemisphere was dissected into four regions before tissue solubilization and the regional BUI was determined (Table 3). Since regional blood flow may vary markedly from one region to another (Table 3), the regional BUI must be normalized for regional differences in clearance of the water reference (Table 3). The normalized BUI values for T_3, which represent a kind of clearance data, are shown in Table 3. Regional clearance of T_3 parallels regional blood flow; however, regional differences in BBB permeability to T_3 may also underlie the regional differences in T_3 transport. Moreover, the regional transport studies show clearly that brain uptake of T_3 is via the BBB and not the choroid plexus; although small amounts of choroid tissue are present in whole brain analyses, there is no choroid plexus associated with the colliculi, caudate-putamen, or olfactory bulb samples.

Efflux of T_3 from brain to blood

The BUI of [^{125}I]T_3 increases with time (Fig. 5), reflecting the greater rate of washout of the [^{3}H]water reference relative to T_3 exodus from the brain. Given the known extraction of the water reference at the time points shown in Fig. 5 (10), the T_3 extraction data may be computed from the BUI. A log plot of the T_3 radioactivity in the brain is linear and indicates that T_3 leaves the brain at a rate of $K = 0.25\ min^{-1}$ ($t_{1/2} = 2.8$ min). Since previous data (7, 15) indicate that the metabolism by brain of thyroid hormones during a 4-min period would be negligible, it is assumed that the loss of T_3 radioactivity from the brain represents efflux of T_3 and not a metabolite (Fig. 5); therefore, the E_{eff} may be determined from the K value (see *Materials and Methods*). Given $F = 0.6\ ml\ min^{-1}g^{-1}$ (10) and assuming V is approximately

TABLE 3. Regional BBB transport of [^{125}I]T_3

Region	BUI (%)[a]	Clearance[b]		CBF[c]
		[^{3}H]H_2O	[^{125}I]T_3	
Colliculi (inferior, superior)	47.9 ± 3.4	1.45 ± 0.11	0.69	1.42
Thalamus-hypothalamus	46.2 ± 0.8	1.26 ± 0.08	0.58	1.06
Caudate-putamen	40.5 ± 1.2	1.30 ± 0.07	0.53	1.02
Olfactory bulb	38.9 ± 0.8	1.03 ± 0.11	0.40	0.74

[a] Values given are the mean ± SE (n = 3-4).

[b] Regional clearance for T_3 is calculated from the product of the BUI × regional clearance of [^{3}H]H_2O; the latter was measured directly (10). Values shown are not absolute measurements but are relative indices of clearance.

[c] Cerebral blood flow for the cat brain (19).

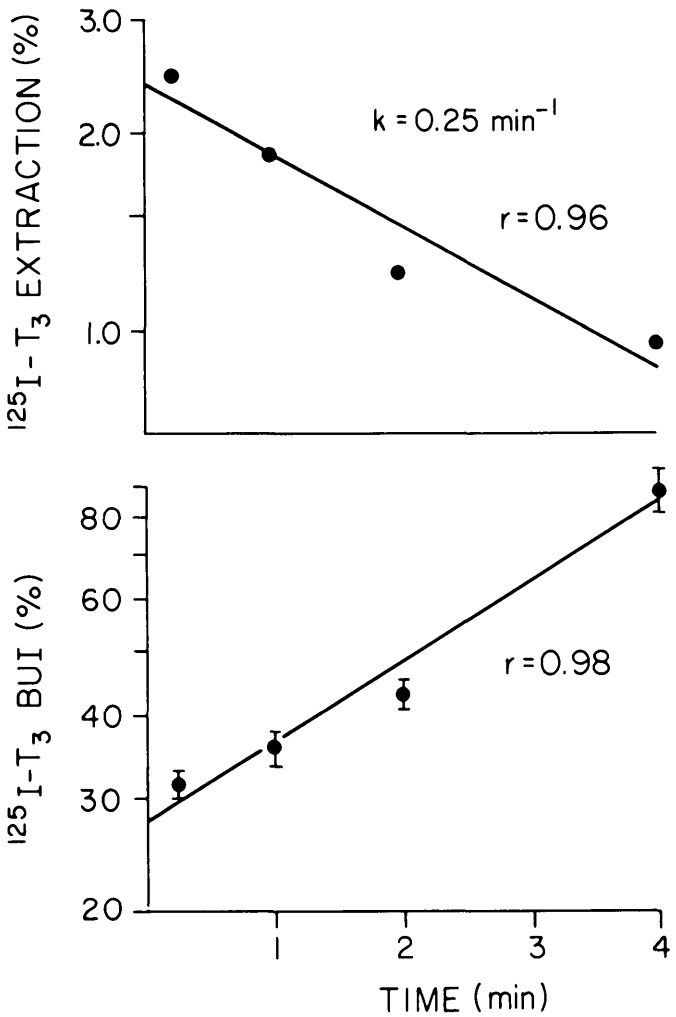

FIG. 5. In the lower figure, the log BUI of T_3 is plotted *vs.* time after carotid injection. Data are the mean ± SE (n = 3-4 rats). The BUI increases with time because the [^{3}H]water reference leaves the brain, after pulse labeling at zero time, faster than the [^{125}I]T_3. The rate of T_3 efflux from brain (K) is obtained from the slope of the *upper panel*, where the log of the percent extraction (or percent of injected dose) of T_3 is plotted *vs.* time after carotid injection. The extraction of T_3 at 0.25, 1, 2, and 4 min was obtained by multiplying the BUI by the known percent extraction (or percent of injected dose) of the [^{3}H]water reference at each time point (10). The data shown are approximately 10% of the actual extraction, since only about 10% of the common carotid bolus goes to the internal carotid while the remaining 90% enters the external carotid (9).

$0.7\ ml\ g^{-1}$ (7), then $E_{eff} = 29\%$. The E_{inf} may be determined from BUI = 31% and $E_R = 75\%$ (10), as described in *Materials and Methods, i.e.* $E_{inf} = 23\%$; therefore, E_{inf} approximates the calculated E_{eff}, suggesting that the BBB permeabilities of both the blood and brain sides of the capillary wall are of a similar magnitude.

Maximal transport capacity (V_{max})

The V_{max} may be calculated from the E_{inf} and K_m values, as previously reported for hexose transport. The

BBB permeability constant (PS; milliliters per g/min), which is equal to the V_{max}:K_m ratio (10), may be calculated using Crone's equation (16), $PS = -F \ln(1 - E_{inf})$; with substitution of $E_{inf} = 0.23$ (calculated from BUI = 31%) and $F = 0.6$ ml $min^{-1}g^{-1}$ (10), $PS = V_{max}:K_m = 0.16$ ml $min^{-1}g^{-1}$. Therefore, V_{max} = (0.16 ml $min^{-1}g^{-1}$) (1.1 μM) = 0.17 nmol $min^{-1}g^{-1}$; this value overestimates somewhat the actual V_{max}, since the E_{inf} value includes the nonsaturable component of T_3 transport (Fig. 2). Since V_{max} = (PS) (K_m) and the PS and K_m values for T_4 are about 2-fold lower and higher, respectively, relative to T_3, the V_{max} values for T_4 and T_3 are comparable.

Discussion

These studies confirm and extend the observation of Hagen and Solberg (7) that a saturable transport system exists in the brain which mediates the uptake of circulating thyroid hormones. In contrast to previous studies (7), these investigations demonstrate conclusively that the transport system lies at the brain capillary wall, *i.e.* the BBB. The affinity of the BBB thyroid hormone transport system for its substrates is the highest among the numerous transport systems known thus far to operate at the BBB (17). For example, the K_m for T_3 (1.1 μM) or T_4 (2.6 μM) is about 100-fold lower than the K_m (160 μM) of tyrosine transport via the neutral amino acid system (12). However, the capacity (V_{max} = 0.1 nmol $min^{-1}g^{-1}$) of T_3 transport is more than 100-fold less than the capacity (V_{max} = 46 nmol $min^{-1}g^{-1}$) of tyrosine transport (12).

Since ligand binding to the transport system is a prerequisite to transport of the substance through the membrane, the thyroid hormone transport system represents a T_3- or T_4-binding system localized at the capillary wall in the brain. Since circulating albumin also binds the thyroid hormones (13), competition for ligand binding between albumin and the BBB transport system may occur at the capillary level. Such competition would be manifested by a discrepancy between the app K_d of albumin binding of T_3 *in vivo vs.* that *in vitro*. The *in vitro* K_d (4.7 μM) of the albumin preparation used in these studies was determined by equilibrium dialysis. The *in vivo* app K_d (76 μM) was found to be 16-fold greater than the real K_d, consistent with competition between albumin and the BBB transport system. The deviation of app K_d from K_d is a function of the binding index of the T_3 transport system, *i.e.* the ratio of the apparent binding capacity (C) to the dissociation constant (K_m) of T_3 binding and transport at the BBB (See *Appendix*), *i.e.* app $K_d = K_d [1 + (C/K_m)]$. In addition to describing the relationship between app K_d and K_d, the above equation is useful in estimating the apparent C for T_3. Given app K_d = 76 μM, K_d = 4.7 μM, and K_m = 1.1 μM, then C = 17 μM.

Given the binding index (BI) of the T_3 transport system, *i.e.* C/K_m, and the binding index of physiological concentrations of albumin, predictions may be made in regard to the fraction (f) of albumin-bound T_3 that is transported into brain, *i.e.* $f = (BI_{BBB}/BI_{BBB} + BI_{ALB})$, were ALB is albumin. Substitution of BI_{BBB} = 17 μM ÷ 1.1 μM = 15 and BI_{ALB} = 600 μM ÷ 4.7 μM = 125 into the above relationship predicts f = 11%, *i.e.* 11% of albumin-bound T_3 will be transported into the brain on a single pass (600 μM albumin is equal to 4.1 g/dl). Since the rat (8) lacks thyroid-binding globulin and only about 1% of rat T_3 is free, *i.e.* dialyzable, as much as 99% of total T_3 in the rat may be albumin-bound. Therefore, the fraction of albumin-bound T_3 that is transported into the brain (11%) is more than 10-fold the free (dialyzable) hormone fraction. These considerations suggest that, in the rat, the major plasma fraction available to the brain is the albumin-bound moiety. The data in Table 2, showing that up to 15% of total T_3 is transported into the brain in the presence of 67% rat serum, corroborate the prediction that about 11% of albumin-bound T_3 is transported. The mechanism by which albumin-T_3 might be transferred from the plasma protein to the transport site is discussed in the *Appendix*.

The fraction of albumin-bound T_4 transported into the rat brain was not measured, but would be expected to be much lower than that for T_3, *e.g.* the BI_{BBB} for T_4 may be 17 μM ÷ 2.6 μM = 6.5, as opposed to the BI_{ALB} = 600 μM ÷ 0.6 μM = 1000 (the albumin K_d for T_4 is about 0.6 μM) (13). Therefore, the predicted f value for T_4 is 6.5/1006 = 0.7%, which is equal to the free (dialyzable) fraction of T_4 in rat plasma (8).

In species such as man where TBG is present in the circulation (13), the fraction of protein-bound T_3 transported into the brain would be reduced for two reasons: 1) only about 30% of circulating T_3 is albumin-bound in man (18), and 2) thyroid-binding globulin represents an additional binding site that will compete with the capillary transport system for T_3 or T_4 binding. Therefore, if albumin-bound T_3 is transported into the brain of man, the binding index, *i.e.* capacity and affinity, of the BBB transport system must be much higher than that observed for the rat. One line of indirect evidence that suggests such a process may occur in man is that the ratio of free T_3 in CSF to free T_3 in plasma is about 5 (18). This suggests that T_3 is either actively transported (against a concentration gradient) into CSF or that the total T_3 in CSF is in equilibrium with a fraction of the protein-bound T_3 in plasma. Based on the high rate of T_3 efflux from the brain (Fig. 5), it is unlikely that the BBB T_3 transport system in the rat actively transports the hormone into the brain from the blood. Therefore, it is possible that the high ratio of CSF free T_3 to plasma free T_3 is due to the transport of protein-bound T_3 into the brain in man.

Finally, the observation that albumin-bound T_3 is transported through the BBB may represent one example of a general phenomenon. For example, albumin-bound tryptophan (20, 21) and albumin-bound steroid hormones (22) are known to be readily transported through the BBB. In addition, protein-bound thyroid hormones (23) and steroid hormones (24) are rapidly transported into the liver. In all cases, the fraction of protein-bound hormone that is transported into tissues *in vivo* greatly exceeds the fraction of ligand that is free (dialyzable) *in vitro*.

Appendix: In Vivo Competitive Ligand Binding Assay

The transport of an albumin-bound ligand such as T_3 is believed to occur via the following mechanism,

$$AL \underset{k_2}{\overset{k_1}{\rightleftharpoons}} A_F + L_F$$

$$+ \underset{k_4}{\overset{k_3}{\rightleftharpoons}} CL \xrightarrow{k_5} C_F + L_M$$

$$C_F$$

where AL and CL are the albumin-ligand and BBB carrier-ligand complexes, respectively; A_F and C_F are free albumin and free carrier; L_F and L_M are free ligand and transported ligand; k_1, k_2, k_4, and k_3 are the dissociation and association constants of the AL and CL complexes, respectively; and k_5 is the rate constant of loaded carrier movement through the membrane. The assumptions of the model are: 1) the ligand dissociation from albumin is an obligatory intermediate in the movement of the ligand between the two binding sites, and this process occurs within the capillary transit time (T), *i.e.* $k_1 > T^{-1}$; 2) both binding systems are in steady state, *i.e.* $k_2 > k_1$ so that $d(AL)/dt = 0$ and $k_3 > k_4$ so that $d(CL)/dt = 0$; 3) the transport K_m represents the dissociation constant of the CL complex, *i.e.* $k_4 > k_5$ such that $K_m = k_4/k_3$; and 4) the carrier movement through the membrane is faster than the capillary transit time, *i.e.* $k_5 > T^{-1}$. Summarizing the above assumptions, $k_2 > k_1 > T^{-1}$ and $k_3 > k_4 > k_5 > T^{-1}$. Since $d(AL)/dt = k_2 (A_F) (L_F) - k_1 (AL) = 0$ and $d(CL)/dt = k_3 (C_F) (L_F) - k_4 (CL) - k_5 (C_L) = 0$, then $(AL) = [(A_F) (L_F)/K_d]$ and $(CL) = [(C_F) (L_F)/K_m]$, where $K_d = k_1/k_2$ and $K_m = (k_4 + k_5)/k_3 = k_4/k_3$. The ligand conservation equation is $L_T = L_F + AL + CL$, where L_T is the total ligand concentration. Substituting AL and CL into the ligand conservation equation and rearranging the terms results in $(AL/L_T) = [A_F/K_d (1 + C_F{:}K_m) + A_F]$, where $AL/L_T = B$ (Fig. 4), *i.e.* the fraction of ligand bound to albumin in the presence of the competing binding system. The double reciprocal plot is $1/B = 1 +$ app K_d $(1/A_F)$, where app $K_d = K_d (1 + C_F{:}K_m)$, as described in the text. Although these relationships are based on free albumin and carrier concentrations, the free values are approximated by the total albumin and carrier concentrations, since the concentration of labeled T_3 (2 nM) $\ll K_d$ or K_m of the two binding systems.

The above equation for app K_d predicts that if the activity of the capillary transport system is sufficiently low, such that $C{:}K_m \ll 1$, then app $K_d = K_d$, and only the free (dialyzable) hormone would be transported into the tissue. However, if $C{:}K_m \geq 1$, then the *in vivo* app K_d will deviate from the *in vitro* K_d in proportion to the $C{:}K_m$ ratio.

Although verification of the assumptions upon which the above model is based would require measurement of the individual rate constants, there are indirect data that suggest the above four assumptions are valid. Firstly, Hillier (25) has shown that the half-time of T_3 dissociation from albumin (k_1) is less than 1 sec, which is less than the mean transit time through the rat brain (T = 2–3 sec) (26). Secondly, the steady state assumption is valid, even for a single injection technique, since rates of amino acid transport determined with the carotid injection technique (27) correlate highly (r = 0.8–0.9) with transport rates obtained by a constant infusion technique (28). Thirdly, evidence that the K_m of T_3 transport is the true dissociation constant, in addition to being a half-saturation constant, comes from the observation that the V_{max} values of T_3 and T_4 transport are essentially the same (see *Results*). A constant V_{max} among different substrates transported by a given transport system indicates that the rate-limiting step of transport is independent of substrate structure, *i.e.* the movement of the CL complex is rate limiting, not the ligand-carrier binding step (29). Finally the CL complex must move through the membrane faster than the bolus transit time, otherwise, labeled T_3 would be eluted off of the capillary wall by the circulating plasma proteins in the approximate 10-sec period between passage of the bolus through the brain and decapitation at 15 sec after injection; the bolus is through the brain within 5 sec after injection (30).

The above mechanism for tissue uptake of thyroid hormones combines essential features of the two major models previously proposed for the transport of thyroid hormones. Hillier (25) has argued that protein-bound thyroid hormones must first dissociate into the free state before entry into the tissue; however, Hillier (25) did not consider the role of competition between plasma protein and cell membrane-binding sites in determining to what extent thyroid hormones enter the tissue *vs.* reassociation with plasma proteins. Conversely, Oppenheimer and associates (31) proposed a collision model, which suggested that protein-bound hormone was transferred directly from plasma protein to tissue-binding sites without an obligatory dissociation into the free state. While little evidence appears to exist in favor of such a collision mechanism, the Oppenheimer model (23, 31) stressed the fundamental role of the competition between plasma

protein and tissue-binding sites in determining the extent of protein-bound hormone transport into tissues.

Acknowledgments

Lawrence Mietus provided skillful technical assistance and Charlotte Limberg prepared the manuscript. The author is indebted to Dr. Inder J. Chopra for numerous and invaluable discussions.

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