0013-7227/87/1213-1185$02.00/0
Endocrinology

Vol. 121, No. 3
Printed in U.S.A.

Stereospecificity of Triiodothyronine Transport into Brain, Liver, and Salivary Gland: Role of Carrier- and Plasma Protein-Mediated Transport*

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ABSTRACT. The stereospecificity of T_3 transport through the walls of the brain capillary, *i.e.* the blood-brain barrier (BBB), and the salivary gland capillary and through the hepatocyte plasma membrane was studied with a tissue-sampling single injection technique in rats. In the absence of plasma proteins, the ED_{50} of inhibition of the saturable transport of [^{125}I]L-T_3 through the BBB was 1 μM for unlabeled L-T_3 and 9 μM for unlabeled D-T_3. The brain extraction of [^{125}I]D-T_3, 5.9 ± 0.1% (±SE), was about one third that of [^{125}I]L-T_3. Conversely, no saturable and no stereospecific T_3 transport was observed for the salivary gland capillary, which, unlike the brain capillary, is porous. The hepatic extraction of T_3 was minimally stereospecific in the absence of plasma proteins. In the presence of 5 g/dl bovine albumin, the fraction of circulating D- or L-T_3 that was available for transport into liver (50–100%) was many-fold greater than the fraction that was free *in vitro* (~2%); therefore, both D-T_3 and L-T_3 were available for uptake by liver from the circulating albumin-bound pool. This plasma protein-mediated transport of T_3 is believed to represent a process of enhanced dissociation of T_3 from the albumin-binding site, since the plasma protein *per se* is not significantly taken up by liver on a single pass. However, in the presence of 5 g/dl bovine albumin, the extravascular hepatic extraction of [^{125}I]D-T_3 (50 ± 2%) was nearly half that for [^{125}I]T_3 (93 ± 12%), although no significant difference in the *in vitro* binding of [^{125}I]D-T_3 and [^{125}I]L-T_3 to 5 g/dl bovine albumin was found with equilibrium dialysis. In addition, the isoelectric point of bovine albumin bound to [^{125}I] L-T_3 (5.1) was higher than that of bovine albumin bound to [^{125}I] D-T_3 (5.0), as determined by isoelectric focusing, which indicates that the surface of the bovine albumin molecule is slightly more positive when the protein binds L-T_3 as opposed to D-T_3. The isoelectric focusing and *in vivo* transport data together suggest that the interaction between the surfaces of the plasma protein and the hepatic microcirculation that is presumed to cause enhanced hormone dissociation from the protein-binding site is electrostatic in nature. These studies (1) show that the BBB thyroid hormone transport system is sharply stereospecific, and this property is probably a major factor underlying the low biological potency of D-T_3 in brain; and (2) provide the first evidence for stereospecificity of plasma protein-mediated transport of hormones into tissues *in vivo*. (*Endocrinology* **121**: 1185–1191, 1987)

THE biological potency of D-T_3 is 3–10 times less than that of L-T_3 (1–4). However, the affinity of D-T_3 for the nuclear receptor that mediates T_3 action (5, 6) is equal to or slightly less than that of L-T_3 (7–9). Since T_3 nuclear receptor occupancy is also a function of the relative rates of cellular uptake of circulating isomers of T_3, stereospecific transport mechanisms for T_3 may provide the major locus of hormone stereospecificity. Since T_3 is avidly bound by plasma proteins (10), there are two major sites where stereospecific transport mechanisms may occur. The first site is the plasma membrane. Previous studies have shown that T_3 enters the brain via a carrier-mediated transport mechanism localized within the brain capillary wall, *i.e.* the blood-brain barrier (BBB) (11). The operation of a similar transport system in liver seems less likely, since previous studies have shown that T_3 transport into liver cells either *in vivo* (12) or *in vitro* (13) is consistent with free diffusion of this relatively lipid-soluble hormone. A second potential site of transport stereospecificity is the T_3-binding site on plasma proteins, such as albumin. Previous studies have shown that T_3 is readily available for transport into liver from the circulating albumin-bound pool (12). Since the albumin molecule *per se* is not significantly taken up by liver on a single circulatory passage (14), the transport of T_3 into liver from the albumin-bound pool is believed to arise from a mechanism of enhanced dissociation of T_3 from the albumin-binding site (15–17). That is, owing to transient interactions between amino acid residues on the surface of the albumin molecule and components of the microcirculation surface, conformational changes in the binding

Received March 4, 1987.

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* This work was supported by NIH Grant RO1-AM-25744. Presented at the Annual Meeting of the American Association of Pharmaceutical Scientists, Washington, D.C., November 1986, and at the Western Section AFCR, Carmel, CA, February 1987.

site ensue, which result in markedly increased rates of hormone dissociation from the plasma protein *in vivo* compared to what occurs *in vitro*. The overall surface charge of the albumin molecule, which is a function of its conformational state, may be a stereospecific function of the ligand bound to albumin. Within the context of this model, plasma protein-mediated T_3 transport into tissues such as liver may show stereospecificity. Therefore, the purpose of the present study was to examine the stereospecificity of carrier-mediated T_3 transport into brain, liver, and salivary gland and the potential stereospecificity of albumin-mediated transport of T_3 into liver *in vivo*. Salivary gland was examined as a control peripheral tissue, since the capillaries in this organ (18), in contrast to those in the brain (19), are porous and allow for free diffusion of T_3 into the tissue.

Materials and Methods

Materials

Unlabeled L-T_3, L-T_4, D-T_3, Sephadex G-25 (fine), *N,N,N′,N′*-tetramethylethylenediamine, and glycerol were purchased from Sigma Chemical Co. (St. Louis, MO). Acrylamide, *N,N′*-methylene-bis-acrylamide, and ammonium persulfate were purchased from Bio-Rad Laboratories (Richmond, CA). Pharmalyte (pH 3–10 and pH 4–6.5) was purchased from Pharmacia Fine Chemicals (Piscataway, NJ). Ultrofilm ^3H was purchased from LKB (Gaithersburg, MD). Kodak GBX developer and fixer were purchased from Eastman Kodak Co. (Rochester, NY). BSA (Pentex, fraction V) was purchased from Miles Laboratories (Elkhart, IN). Serum was obtained from ketamine/xylazine-anesthetized rats. All other reagents were commercially available.

All isotopes except [^{125}I]D-T_3 were obtained from New England Nuclear (Boston, MA). The specific activities of the isotopes are as follows: [^{125}I]L-T_3, 150 μCi/μg; [^{125}I]L-T_4, 150 μCi/μg; [^{125}I]iodine, 2.17 mCi/nmol; and ^3H_2O, 1 mCi/g.

Iodination of D-T_3

D-T_3 was iodinated with [^{125}I]iodine to a specific activity of 100 μCi/μg with the use of chloramine-T. The iodination was carried out by adding sequentially, to a glass tube, 7 μl 1 mg/ml D-T_3, 10 μl 0.3 M sodium phosphate buffer (pH 7.4), 10 μl 500 mCi/ml [^{125}I]iodine, and 10 μl 100 μM chloramine-T. The mixture was incubated for 1 min at room temperature, and 200 μl 0.1 M sodium acetate (pH 5.6) were added. The solution was then transferred to a 0.7 × 18-cm Sephadex G-25 (fine) column. The column was eluted with 16 ml 0.1 M sodium acetate (pH 5.6) and then with 22 ml 0.1 N NaOH. Initially, the column was eluted with 12 ml 0.1 M sodium acetate (pH 5.6), but this incompletely removed free iodine (Fig. 1). Elution with 16 ml acetate buffer removed any detectable iodine contamination in the [^{125}I]D-T_3 preparation, as detected by TLC (see below).

The radiochemical purities of [^{125}I]L-T_3, [^{125}I]L-T_4, and [^{125}I]D-T_3 were at least 99%, as judged by cellulose (250 μm) TLC and radiochromatogram scanning (model 7230 radiochromatogram scanner, Packard, Downers Grove, IL) using the solvent system of chloroform-*t*-butanol-2 N NH_4OH (60:376:70). The radiochemical purity of [^{125}I]D-T_3 was also checked by HPLC (model 110A, Altex Scientific, Inc., Berkeley, CA) (20). The analytical column was a reverse phase C_{18} Ultrasphere column (id, 15 cm × 4.6 mm; Altex). The mobile phase was 30% acetonitrile in 0.1 M sodium acetate with 4 mM cupric sulfate and 8 mM L-proline. The solvent flow rate was 1.5 ml/min. The eluate was collected every minute, and each 1.5-ml fraction was counted for ^{125}I.

Tissue-sampling single injection technique

The extraction of unidirectional influx for [^{125}I]D-T_3 and [^{125}I]L-T_3 relative to a ^3H_2O reference was measured by the tissue-sampling single injection technique in adult male Sprague-Dawley rats (240–290 g) under ketamine/xylazine anesthesia (ketamine, 235 mg/kg, im; xylazine, 2.3 mg/kg, im). An approximately 200-μl bolus of HEPES-buffered Ringer's solution (pH 7.4, RHB) was rapidly injected (<1 sec) via a 27-gauge needle into the common carotid artery for the BBB transport (11) and salivary gland transport studies (18) or into the portal vein after ligation of the hepatic artery for the hepatic transport study (12). The injection vehicle contained [^{125}I]D-T_3 or [^{125}I]L-T_3 (2 μCi/ml), ^3H_2O (20 μCi/ml) and various concentrations of albumin or serum in RHB. Trace concentrations of BSA (0.05–0.1%) were added to the RHB solution to prevent T_3 adsorption to the injection syringe. This concentration of BSA causes no inhibition of T_3 transport into brain or liver (11, 12). Mixing of the injectate with the circulating blood has been found to be less than 5% (21). In the case of BBB and salivary gland transport studies, the rat was decapitated 15 sec after carotid injection, and then the hemisphere and the mandibular gland ipsilateral to the injection site were removed. Eighteen seconds after the injection, the portal vein was transsected, and the right major lobe of liver was rapidly removed for the hepatic transport study. Portions of brain, salivary gland, and liver were solubilized in 2.0 ml Soluene-350 (Packard) at 50 C for 1 h before double isotope liquid scintillation counting.

Counts per min were converted to disintegrations per min by standard quench corrections, and the percent brain uptake index (BUI), salivary gland uptake index (SUI), and liver uptake index (LUI) were calculated as follows:

$$\text{BUI, SUI, or LUI} = \frac{(^{125}\text{I dpm}/^{3}\text{H dpm})\ \text{tissue}}{(^{125}\text{I dpm}/^{3}\text{H dpm})\ \text{injectate}} \times 100$$

The percent extractions of T_3 in brain (E_b), salivary gland (E_s), and liver (E_l) were calculated: $E_b = BUI \times E_{rb}$, $E_s = SUI \times E_{rs}$, and $E_l = LUI \times E_{rl}$, where E_{rb}, E_{rs}, and E_{rl} represent the fractional brain, salivary gland, and liver extractions of ^3H_2O, respectively. E_{rb}, E_{rs}, and E_{rl} are 0.64, 0.70, and 0.84, as previously reported (16, 18, 22, 23). Although the vascular volume relative to whole tissue volume of brain is so small that it is negligible for the calculation of extravascular brain extraction (19), the vascular volume of salivary gland and liver is not negligible (12, 18). Therefore, the percent extraction by salivary gland ($E_{x,s}$) and liver ($E_{x,l}$) due to extravascular uptake of T_3

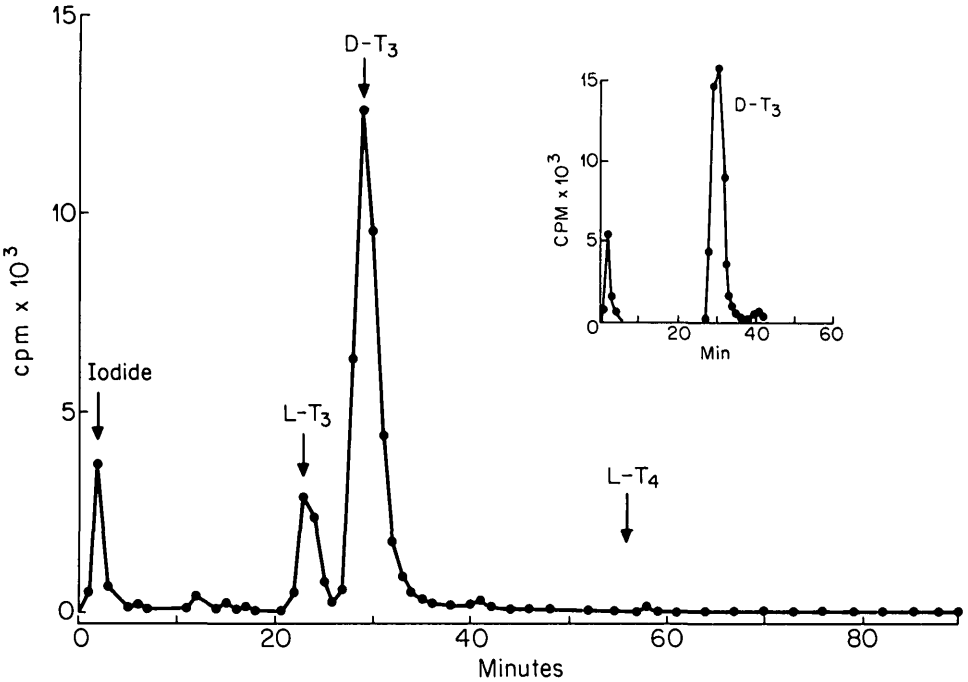

FIG. 1. Chromatogram of the enantiomers of [^{125}I]T_3. The mixture of 0.1 μCi [^{125}I]L-T_3 and 0.5 μCi [^{125}I]L-T_3 was injected into the reverse phase C_{18} column. The eluate was collected at 1-min intervals. The separation conditions were as follows: mobile phase, 30% acetonitrile in 0.1 M sodium acetate with 4 mM cupric sulfate and 8 mM L-proline; flow rate, 1.5 ml/min. A chromatogram of the [^{125}I] D-T_3 is illustrated in *inset*.

were calculated:

$$E_{x,s} = \frac{E_s - E_{al,s}}{100 - E_{al,s}} \times 100$$

$$E_{x,l} = \frac{E_l - E_{al,l}}{100 - E_{al,l}} \times 100$$

where $E_{al,s}$ and $E_{al,l}$ represent the percent extraction of albumin in salivary gland and liver, respectively. The $E_{al,s}$ and $E_{al,l}$ values are 17 ± 2% and 13 ± 2%, which were determined previously (12, 18).

In vitro equilibrium dialysis

The *in vitro* percentage of free (dialyzable) [^{125}I]D-T_3 or [^{125}I] L-T_3 in the presence of either sera or albumin was determined by equilibrium dialysis (12). One and one half milliliters of the injectate containing 0.5 μCi/ml [^{125}I]T_3 were applied to a dialysis bag made of a 1.5 × 15-cm strip of dialysis tubing (mol wt cut-off, 12,000–14,000; Spectrum Medical Industries, Inc., Los Angeles, CA) and dialyzed against 15 ml RHB containing 0.05% sodium azide at 37 C for 20 h. The *in vitro* percent free was calculated from the ratio of disintegrations per min/ml in the dialysis buffer to that in the dialysis bag.

Isoelectric focusing (IEF) and autoradiography

IEF was carried out on prefocused horizontal polyacrylamide slab gels over a pH range of 3.8–6.5 at 4 C, with the use of a flatbed electrophoresis apparatus (C.B.S. Scientific Co., Del Mar, CA), an electrophoresis constant power supply (ECPS 3000/150, Pharmacia Fine Chemicals), and a volt-hour integrator (VH-1, Pharmacia Fine Chemicals) (24). Twenty-five milliliters of the gel solution containing 4.875% acrylamide, 0.125% *N,N'*-methylene-bis-acrylamide, 8% glycerol, 1.06 ml Pharmalyte (pH 4–6.5), and 0.6 ml Pharmalyte (pH 3–10) were prepared. After degassing, polymerization was carried out in a casting mold (thickness, 1 mm; width, 140 mm; length, 160 mm) in the presence of 250 μl 3% ammonium persulfate and 28 μl *N,N,N',N'*-tetramethylethylenediamine. Prefocusing was done with an anode solution (25 mM DL-aspartic acid and 25 mM L-glutamic acid) and a cathode solution (250 mM glycine) in a setting of constant power at 9 watts for 60 min, reaching a maximum of 2000 V. Then, 7 μl 5 g/dl BSA containing 10 μCi/ml [^{125}I]D-T_3 or [^{125}I]L-T_3 were applied to the center of the gel. IEF was carried out for 5 h at a constant power of 13 watts, reaching a maximum of 2300 V. Immediately after the current was turned off, the pH gradient in the gel was determined with a pH meter (pH meter 140, Corning Science Products, Medfield, MA). Half-centimeter slices of the gel were eluted in 2 ml 50 mM KCl. The gel was then transferred to the filter paper, covered with plastic film, and dried for 1 h at 60 C on a gel slab dryer (model 224, Bio-Rad Laboratories). The dried gel was exposed to LKB Ultrofilm for 2 days at room temperature and then developed by Kodak GBX developer for 4 min. The development was stopped by 2% acetic acid for 30 sec and washed in water for 30 sec. Then, the film was fixed with Kodak GBX fixer for 5 min, followed by a wash with water for 20 min and complete drying.

Statistical differences were assessed by Student's *t* test.

Results

The chromatogram of a mixture of [^{125}I]L-T_3 and [^{125}I] D-T_3 is shown in Fig. 1. The retention times of [^{125}I]L-T_3, [^{125}I]D-T_3, and [^{125}I]L-T_4 were 23, 29, and 56 min, respectively. The typical chromatogram of the [^{125}I]D-T_3, which was prepared by the chloramine-T iodination method, is also shown in the *inset* of Fig. 1. The small

amount of free iodine found in the HPLC run after elution of the G-25 column with 12 ml acetate buffer (Fig. 1) was not found on subsequent HPLC runs after elution of the G-25 column with 16 ml acetate buffer (see *Materials and Methods*). The HPLC system separated the D- and L-isomers of T_3 (Fig. 1), and no [^{125}I]L-T_3 was found in the [^{125}I]D-T_3 preparation (Fig. 1, *inset*).

The concentration dependency for the brain extraction of [^{125}I]L-T_3 is illustrated in Fig. 2. The nonsaturable brain extraction (5.86 ± 0.02%) was obtained from the extraction of [^{125}I]L-T_3 in the presence of 100 μM L-T_3. The ED_{50} for the saturable brain extraction of L-T_3 was 1 μM. The inhibition of the brain extraction of [^{125}I]L-T_3 by D-T_3 is also illustrated in Fig. 2. The ED_{50} of D-T_3 inhibition of [^{125}I]L-T_3 extraction was 9 μM. The brain extraction of [^{125}I]D-T_3 in RHB with 0.05 g/dl BSA is compared to that of [^{125}I]L-T_3 in the *inset* of Fig. 2. The brain extraction of [^{125}I]D-T_3 was significantly less than that of [^{125}I]L-T_3, and no difference was observed between the brain extraction of [^{125}I]D-T_3 (5.78 ± 0.35%) and the nonsaturable extraction of [^{125}I]L-T_3 (5.86 ± 0.07%).

The extractions of [^{125}I]D-T_3 and [^{125}I]L-T_3 by rat salivary gland were also examined (Table 1). No significant difference was observed between extravascular salivary gland extraction of [^{125}I]D-T_3 and [^{125}I]L-T_3 in the presence of 0.05 g/dl BSA. Additionally, there was no significant difference in the extraction of [^{125}I]L-T_3 in the absence or presence of 25 μM L-T_3 and 25 μM D-T_3 in the injectate. Furthermore, no significant difference was found for the extraction of [^{125}I]D-T_3 in the absence or presence of 25 μM D-T_3 in the injectate (Table 1).

When plasma proteins were essentially absent from the portal venous injection vehicle (*e.g.* 0.1 g/dl BSA; Table 2), the extravascular hepatic extraction of L-T_3 was only 12% greater than that for D-T_3. The effect of serum protein binding on hepatic extraction of L-T_3 and D-T_3 was investigated by adding 5 g/dl BSA or 92% rat serum to the injection solutions (Table 2). The extravascular hepatic extraction of [^{125}I]D-T_3 in the presence of 5 g/dl BSA was significantly less than that of [^{125}I]L-T_3 ($P < 0.05$). On the contrary, there was no difference between the extravascular extraction of [^{125}I]D-T_3 and [^{125}I]L-T_3 in the presence of 92% rat serum (Table 2).

The *in vitro* binding of [^{125}I]D-T_3 and [^{125}I]L-T_3 to 5 g/dl BSA and 100% rat serum was determined by the equilibrium dialysis technique. The *in vitro* unbound percentages (dialyzable fraction) of [^{125}I]D-T_3 and [^{125}I]L-T_3 in the presence of 5 g/dl BSA were 1.95 ± 0.16% and 1.79 ± 0.28%, respectively, and these values were not significantly different. The *in vitro* unbound percentages of [^{125}I]D-T_3 and [^{125}I]L-T_3 in the presence of 100% rat serum were 4.30 ± 0.31% and 2.42 ± 0.28%, respectively ($P < 0.01$).

The isoelectric point (pI) of BSA was determined by sequential IEF and autoradiography in the presence of 5 g/dl BSA bound by either [^{125}I]L-T_3 of [^{125}I]D-T_3. As shown in Fig. 3, the pI value of BSA bound to [^{125}I]L-T_3 was 5.1, which was greater than that of BSA bound to [^{125}I]D-T_3 (pI 5.0).

Discussion

The present studies provide evidence for 1) stereospecific carrier-mediated transport of T_3 through the brain capillary wall, *i.e.*, the BBB; 2) nonstereospecific transport of T_3 through the salivary gland capillary wall and essentially nonstereospecific transport of T_3 through the

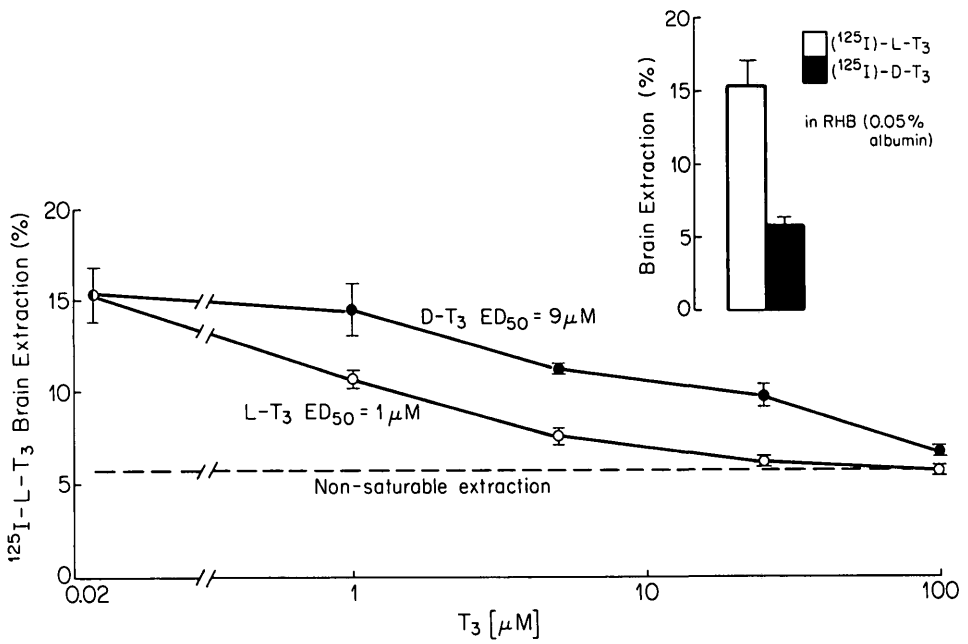

FIG. 2. The brain extraction of [^{125}I]L-T_3 is decreased by the presence of L-T_3 or D-T_3 in the carotid injection solution. O, The brain extraction of [^{125}I]L-T_3 inhibited by unlabeled L-T_3. ●, The brain extraction of [^{125}I]L-T_3 inhibited by unlabeled D-T_3. The nonsaturable extraction (5.86 ± 0.02%) represents the extraction of [^{125}I]L-T_3 after injection of labeled hormone in 100 μM L-T_3. The brain extractions of [^{125}I]D-T_3 and [^{125}I]L-T_3 after injection of isotope mixed in RHB containing 0.05 g/dl BSA are compared in the *inset*. Data are the mean ± SE for three experiments.

TABLE 1. Extravascular salivary gland extraction of [^{125}I]D-T_3 and [^{125}I]L-T_3

Injection vehicle (RHB)	Extravascular salivary gland extraction (%)	
	D-T_3	L-T_3
0.05% albumin	30.3 ± 4.1	34.2 ± 2.4
25 μM L-T_3		33.7 ± 2.2
25 μM D-T_3	30.3 ± 2.0	29.0 ± 1.8

Values given are the mean ± SE (n = 3).

TABLE 2. Comparison of rat extravascular hepatic extraction of [^{125}I] D-T_3 and [^{125}I]L-T_3 in the presence of various serum proteins

Injection vehicle[a]	Extravascular hepatic extraction (%)	
	D-T_3	L-T_3
0.1% BSA	73.4 ± 1.1	84.3 ± 3.7[b]
5.0% BSA	49.9 ± 2.2	93.1 ± 11.7[b]
92% Rat serum	55.2 ± 3.8	52.0 ± 1.7

Values given are the mean ± SE (n = 3).

[a] All injectate solutions were diluted with RHB (pH 7.4).

[b] Significantly different ($P < 0.05$, by Student's t test) from D-T_3 hepatic extraction at the same injectate concentration.

hepatocyte cell membrane; 3) biochemical evidence for different albumin conformational states, depending on whether the plasma protein binds L-T_3 or D-T_3; and 4) the first evidence of stereospecificity of plasma protein-mediated transport of albumin-bound ligands into tissues *in vivo*. These results are discussed within the context of 1) plasma membrane T_3 transport in brain, salivary gland, or liver; 2) plasma protein-mediated transport of T_3 in liver; and 3) the implications of these findings to the differential biological potencies of the L- and D-isomers of T_3.

Previous *in vivo* studies have shown that the uptake of T_3 by brain is saturable, whereas T_3 uptake by other organs, such as liver, is nonsaturable (25, 26). Earlier studies from this laboratory have shown that a specific thyroid hormone carrier-mediated transport system is present in the brain capillary wall or BBB (11). The BBB thyroid hormone transport system was found to have a higher affinity for T_3 (K_m = 1 μM) than for T_4 or T_3, and was found to be a distinct entity from the known neutral amino acid transport system that is also active within the BBB (11). The availability of methods for iodinating D-T_3 and for determining the isomeric purity of the iodinated D-T_3 preparation (20) provided the opportunity for assessing the stereospecificity of the BBB thyroid hormone transport system. The present studies show that this carrier has a 9 times higher affinity for L-T_3 than for D-T_3. Owing to the very low affinity of D-T_3 for this transport system, virtually the only transport pathyway for D-T_3 movement through the BBB is free diffusion. For example, the first pass extraction of [^{125}I] T_3 (5.8%) is no greater than the nonsaturable, *i.e.* not carrier mediated, extraction of [^{125}I]L-T_3 (5.9%). The flux of D-T_3 through the nonsaturable mechanism, *i.e.* free diffusion, is about one third of the total influx of L-T_3 through both the nonsaturable and carrier-mediated mechanisms.

In contrast to brain capillaries, which have no pores, salivary gland capillaries are fenestrated (18), and the presence of pores in salivary gland capillaries obviates the need for carrier-mediated transport mechanisms. The absence of a specific T_3 transport system from salivary gland capillaries is supported by the observation that T_3 transport in this organ is neither saturable nor stereospecific.

Hepatic microvessels are sinusoids, which have very large pores and no basement membrane (27). Thus, the sinusoidal wall provides no diffusion barrier to either small molecules, such as free T_3, or plasma proteins, such as albumin. In liver the limiting membrane preventing free diffusion of circulating substances into the hepatocyte cell water is the hepatocyte plasma membrane. Previous studies of T_3 transport *in vivo* have shown that these relatively lipid-soluble hormones undergo rapid nonsaturable transport across the hepatocyte cell membrane (12). Similarly, hepatocyte cell membrane transport of T_3 is weakly, if at all, stereospecific. As shown in

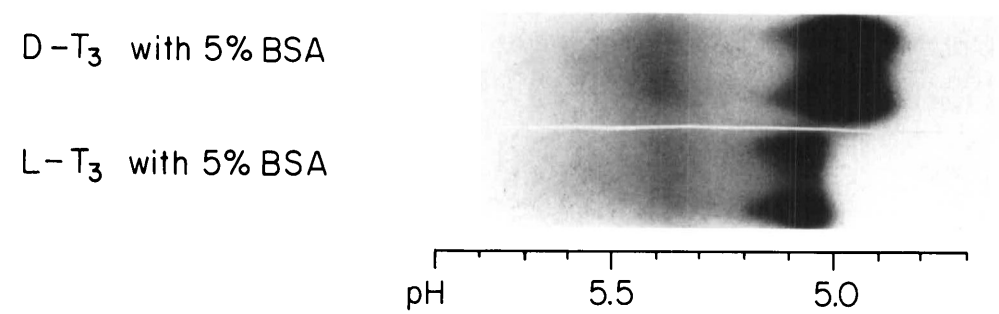

FIG. 3. Autoradiograph of the IEF gel for [^{125}I]L-T_3 and [^{125}I]D-T_3 bound to BSA. The pH of the gel is shown on the *ordinate* of the figure. The content of the sample is shown on the *abscissa*. The concentration of both [^{125}I]D-T_3 and [^{125}I]L-T_3 were 2 μCi/ml, and the albumin concentration was 5 g/dl. The minor band at pH 5.4 represents unbound [^{125}I]L-T_3 or [^{125}I]D-T_3, as this peak comigrated with free [^{125}I]T_3.

Table 2, the extravascular hepatic extraction of D-T_3 (in the presence of 0.1 g/dl BSA) is only 12% lower than the extravascular hepatic extraction of L-T_3. The physiological significance of this difference is questionable, and it is likely that D-T_3 traverses the hepatocyte cell membrane by a nonsaturable nonstereospecific mechanism involving free diffusion.

Although the transport of T_3 across the hepatocyte cell membrane is essentially nonstereospecific, the present studies provide evidence for stereospecificity of albumin-mediated transport of T_3 into liver *in vivo*. In these experiments, liver was used to assess the stereospecificity of plasma protein-mediated transport, since the uptake of T_3 by liver is several times larger than the uptake of T_3 by the brain or the salivary gland. The high hepatic extraction facilitates the measurement of plasma protein-binding effects *in vivo*. In the presence of 5 g/dl BSA, the extravascular hepatic extraction of D-T_3 was about half that of L-T_3, whereas the *in vitro* albumin binding of D-T_3 was comparable to that of L-T_3 (*cf. Results*). A similar pattern was also found for rat albumin. *In vitro*, binding of L-T_3 to rat serum was nearly twice as great as that of D-T_3 (*cf. Results*). However, the *in vivo* extravascular hepatic extractions of D-T_3 and L-T_3 in the presence of rat serum were not significantly different. Therefore, there are species differences in T_3 binding to albumin *in vivo* and *in vitro*. T_3 binding to BSA is stereospecific *in vivo* but not *in vitro*, whereas T_3 binding to rat albumin is stereospecific *in vitro* but not *in vivo*. Since the stereospecific binding of T_3 to albumin is a function of the conformation about the protein binding site, these studies suggest that the T_3-binding site on albumin is different *in vivo* in the liver microcirculation compared to *in vitro*.

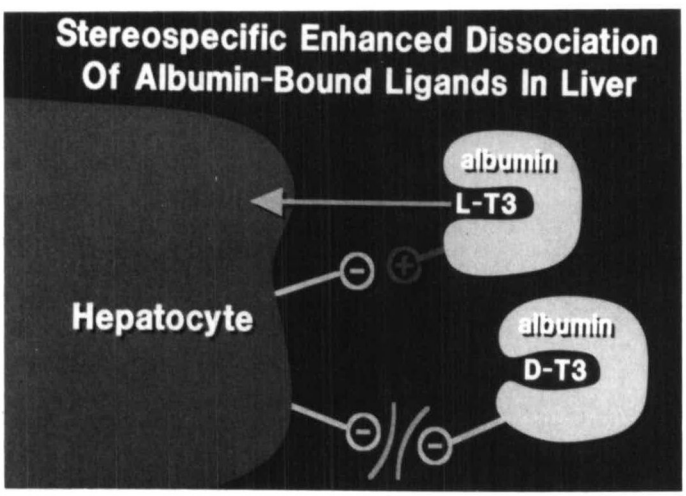

FIG. 4. Hypothetical charge selectivity mechanism for stereospecific enhanced dissociation of albumin-bound T_3 within the liver microcirculation. Albumin carrying L-T_3 is slightly more basic than albumin carrying D-T_3 (see Fig. 3), and L-T_3 is more readily available for uptake by hepatocytes from the circulating albumin-bound pool than is albumin-bound D-T_3 (see Table 2). Therefore, it is proposed that an electrostatic interaction between a positively charged residue on the surface of the albumin molecule carrying D-T_3 and a negatively charged residue on the hepatocyte surface causes a conformational change in the T_3-binding site that results in enhanced dissociation of T_3 from albumin that is preferential for the L-T_3 isomer.

Stereospecificity differences in L-T_3 *vs.* D-T_3 binding to albumin *in vivo* may provide insight into the mechanism of plasma protein-mediated T_3 delivery to liver. The amount of circulating L-T_3 or D-T_3 available for uptake by liver *in vivo* was many times greater than the fraction of T_3 that was unbound *in vitro*. Therefore, circulating T_3 is readily available for uptake by liver from either the circulating bovine or rat albumin-bound pools. Since the plasma protein *per se* is not significantly taken up by liver on a single circulatory passage (14), the transport of hormone from the circulating albumin-bound pool represents a process of enhanced hormone dissociation from the albumin-binding site (15–17). It is hypothesized that transient interactions between the surface of the albumin molecule and the surface of the hepatocyte cause conformational changes in the T_3-binding site on the albumin molecule that result in a binding site uncoiling and markedly increased rates of T_3 dissociation from albumin *in vivo* in the liver microcirculation, but this does not occur *in vitro*. Biochemical evidence in support of the tissue-mediated conformational change in hormone-binding proteins has been recently reported (28). The present studies provide additional biochemical evidence for this model and suggest that the basis for the interaction between albumin and the hepatic microcirculatory surface is an electrostatic one. The IEF data in Fig. 3 show that the surface charge of albumin is a stereospecific function of the nature of the bound ligand. The albumin surface charge is slightly but significantly less negative when the plasma protein is carrying L-T_3 compared to D-T_3. Thus, the binding of L-T_3 to albumin may allow for the exposure of an important lysine or arginine residue on the surface of the plasma protein that is not completely exposed when D-T_3 is bound to albumin (see model in Fig. 4). The data in Table 2, showing the restricted transport of albumin-bound D-T_3 compared to albumin-bound L-T_3, indicate that there is a greater uncoiling around the T_3-binding site on albumin when the plasma protein assumes the conformation that yields the less negative surface charge. The charge selectivity model shown in Fig. 4 is also consistent with the finding by other workers that plasma proteins such as albumin normally undergo rapid electrostatic interactions with the cellular surface of the microcirculation (29, 30).

Finally, in regard to the difference in biological potency between D- and L-T_3, the present studies indicate that membrane transport mechanisms provide marked stereospecificity for the T_3 stereoisomers in brain, but not in

salivary gland or liver. The marked stereospecific differences between D- and L-T_3 transport through the BBB, however, may explain a large component of the overall differences in biological potency of the two stereoisomers of T_3, if T_3 action in the brain regulates organ function in organs other than the brain. For example, Goldman and co-workers (31) have recently shown that intracerebral administration of T_3 to hypothyroid rats results in a greater increase in heart rate than when an equal dose of T_3 is administered iv. Thus, the acceleration of bradycardia in hypothyroidism after T_3 administration may actually represent T_3 action in brain, rather than direct T_3 action on the myocardium. Not all of T_3 actions are mediated via the central nervous system. However, those functions in peripheral organs that are regulated by brain would be expected to show stereospecific differences in the biological potencies of D- and L-T_3, since these two stereoisomers are transported through the BBB at markedly different rates.

Acknowledgments

The authors thank Jody Eisenberg for her excellent technical assistance, Dawn Brown for her skillfull preparation of the manuscript, Billy Joe Green of Abbott Laboratories for assistance in iodination of D-T_3, and Dr. Colum Gorman and his associates for discussions regarding the HPLC separation of the T_3 stereoisomers.

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