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# BLOOD-BRAIN TRANSPORT OF TRIIODOTHYRONINE IS REDUCED IN AGED RATS*

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SUMMARY

An age-related decline in blood-brain barrier transport of thyroid hormones may contribute to the central nervous system changes with aging. To test this hypothesis, the brain uptake index (BUI) of levo (L) and dextro (D) triiodothyronine ($T_3$) was determined in male Fischer 344 rats at 6 months of age (young) and 26 months of age (aged). Young rats pair fed with aged were included to control for reduced food intake in aged rats. The L-$T_3$ BUI of aged rats ($22.4 \pm 2.1\%$) was significantly reduced compared to young rats ($29.5 \pm 2.0\%$) or young rats pair fed with aged rats ($28.5 \pm 2.5\%$) ($p<0.05$). This could not be attributed to age-related changes in BBB permeability or to reduced cerebral blood flow. At steady state conditions, the brain uptake of either L-$T_3$ or D-$T_3$ was not altered with aging. There were no significant changes in plasma or brain binding of $T_3$. These results indicate that the reduced BBB transport of $T_3$ in aged rats is counterbalanced by a reduction in $T_3$ clearance from the brain.

*Key words*: Blood-brain barrier; Triiodothyronine transport; Aging

## INTRODUCTION

It is generally recognized that there is substantial overlap between the age-associated changes and the clinical manifestations of hypothyroidism [10]. Of particular interest is the slow mentation that accompanies both aging and hypothyroidism. Whether this similarity is coincidental or is secondary to a subtle age-related tissue hypothyroidism remains unknown. One possible

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mechanism of age-related reduction in tissue responsiveness to thyroid hormones is reduced tissue uptake of the hormone *in vivo*. Previous studies have documented significant age-related changes in the transport function of the blood-brain barrier (BBB) [8,9]. To determine if the BBB transport of thyroid hormone is altered with age, the brain uptake of levo (L) and dextro (D) enantiomers of triiodothyronine ($T_3$) was determined both at steady state conditions and during the first 15 s following carotid artery injections.

## MATERIALS AND METHODS

Male Fischer 344 rats were obtained from the National Institute of Aging Colony which is maintained by Harlan Laboratories, Indianapolis, Indiana. Six-month-old animals (young) were compared to 26-month-old animals (aged). These animals had free access to food (Purina Rat Chow; Ralston Purina Co., St. Louis, MO) and water. To control for the reduced food intake in the aged rats, an additional group of young rats pair fed with aged rats for 3 weeks was studied.

L-[$^{125}$I]$T_3$ (spec. act. 150 $\mu$Ci/$\mu$g) and tritiated water (spec. act. 1 mCi/ml) were purchased from New England Nuclear, Boston, MA. D-[$^{125}$I]$T_3$ (spec. act 568 $\mu$Ci/$\mu$g) was custom prepared by Abbott Laboratories (North Chicago, IL). The radiochemical purity of [$^{125}$I]$T_3$ was more than 95% by paper chromatography using amyl alcohol-hexane-ammonia solvent system [1]. The enantiomeric purity of D-$T_3$ was 98% as determined by the supplier using high performance liquid chromatography [5]. Tracer doses (<5 ng/100 g body wt) were injected through the tail vein. Animals were killed at intervals from 5 min to 24 h. Blood was collected from the abdominal aorta under light enflurane anesthesia. Plasma [$^{125}$I]$T_3$ concentrations were then determined in TCA-treated samples. The radioactivity was expressed as percent of dose injected per 1 ml of plasma (% dose/ml). The brain tissue was accurately weighed and radioactivity determined as percent of dose injected per gram of tissue (% dose/1 g). Previous studies have shown that [$^{125}$I]$T_3$ accounts for more than 90% of the total tissue radioactivity [14] and therefore no correction is necessary when calculating tissue/plasma isotopic concentration ratios.

To determine the effect of aging on tissue binding of $T_3$, equilibrium dialysis of brain homogenate was done using a previously described method [11,15]. Plasma was diluted ten-fold with 0.15 M sodium phosphate buffer (pH 7.4) and 0.1 $\mu$Ci of [$^{125}$I]$T_3$ was added. Duplicate 2-ml aliquots were dialyzed for 18–20 h at 37°C against 3 vols. of the same buffer. Brain homogenate was diluted 20 times before the equilibrium dialysis and 1 ml of homogenate was dialyzed against 8 vols. of the same buffer. The dialysis fraction, DF, a measure of tissue $T_3$ binding, was calculated as the ratio of total diffusible counts per minute (cpm) to the sum of cpm inside the dialysis bag and the diffusible cpm.

To determine the effect of age on the initial blood-brain transport of

$T_3$, single arterial injection-tissue uptake technique of Oldendorf and Braun was used [13]. Under pentobarbital anesthesia (50 mg/kg i.p.) a total volume of 0.2 ml of 10 mM HEPES-buffered (pH 7.4) Ringer's saline containing 1.25 $\mu$Ci/ml of L-[$^{125}$I]$T_3$, 12.5 $\mu$Ci/ml of $^3H_2O$ and 0.025% of bovine serum albumin was injected in the right common carotid artery, and 15 s later the animal was decapitated. The ipsilateral forebrain was removed, the tissue was solubilized in 1.5 ml soluene (Packard Instrument Co.) at 50°C for 2 h, 10 ml of scintillation cocktail (Instagel, Packard Instrument Co.) was added and the radioactivity was counted in a Beckman liquid scintillation counter. Counts per minute (cpm) recorded were converted to true disintegrations per minute (dpm), with appropriate quench corrections were made as described previously [17]. Brain uptake index (BUI) was determined as

$$\text{BUI} = \frac{(^{125}\text{I dpm}/^{3}\text{H dpm})\text{brain}}{(^{125}\text{I dpm}/^{3}\text{H dpm})\text{ injectate}} \times 100$$

The organs of the aged animals were inspected for gross pathology. Animals bearing tumors or enlarged spleens were excluded from the analysis. The experiments in aged rats and young rats were carried out concurrently. All the results are presented as mean ±S.E.M. Student's *t*-test for unpaired variables was used for the statistical analysis.

## RESULTS

Figure 1 shows the time course of $T_3$ uptake by the brain following intravenous

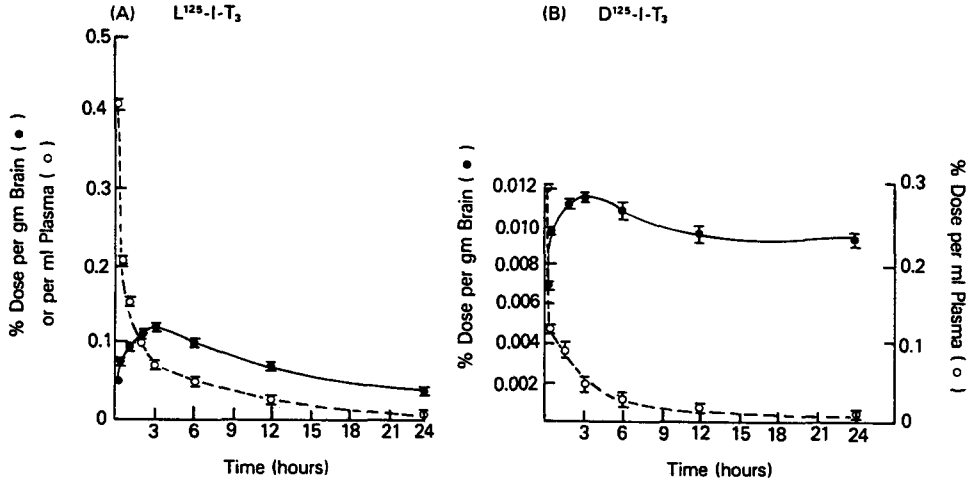


Fig. 1. The time course of plasma radioactive $T_3$ clearance and the time course of radioactive $T_3$ uptake by the brain following intravenous injection of tracer concentrations of L-[$^{125}$I]$T_3$ (A) and D-[$^{125}$I]$T_3$ (B). Each point is the mean ±S.E.M. of 3–8 rats.

TABLE I

BRAIN/PLASMA ISOTOPIC RATIO (B/P) AS PERCENTAGE OF DOSE/g PER PERCENTAGE DOSE/ml OF L-$T_3$ AND D-$T_3$ IN YOUNG RATS (6 MONTHS OLD), AGED RATS (26 MONTHS OLD) AND YOUNG RATS PAIR FED WITH AGED RATS (6 MONTHS OLD)
L/D is the ratio of L-$T_3$ uptake to D-$T_3$ uptake.

| | *Young rats* ($n = 7$) | *Aged rats* ($n = 7$) | *Young rats pair fed with aged* ($n = 5$) |
|---|---|---|---|
| L-$T_3$ | $1.68 \pm 0.04$ | $1.67 \pm 0.04$ | $1.54 \pm 0.05$ |
| D-$T_3$ | $0.29 \pm 0.03$ | $0.301 \pm 0.02$ | $0.28 \pm 0.01$ |
| L/D ratio | 5.57 | 5.68 | 5.42 |

injection. After initial rise of plasma [$^{125}$I]$T_3$ levels there is a rapid decline within the first 2 h of the $T_3$ administration. Thereafter the [$^{125}$I]$T_3$ levels decrease gradually over the ensuing 24 h of observation. The brain accumulation of [$^{125}$I]$T_3$ proceeds gradually until it reaches its peak at 3 h, thereafter, the time course of brain [$^{125}$I]$T_3$ decline parallels that of plasma [$^{125}$I]$T_3$. Thus the brain distribution of $T_3$ reaches steady states after 3 h of intravenous dose. The time course of D-[$^{125}$I]$T_3$ distribution was similar to that of L-[$^{125}$I]$T_3$. These results are consistent with previously published observations [16]. Table I summarizes the brain/plasma ratio of D-$T_3$ and L-$T_3$ measured at steady state in young rats, aged rats and young rats pair fed with aged rats. There were no significant differences between the three experimental groups. At steady state conditions the stereospecific uptake of $T_3$ by the brain was not altered in aged rats. The lack of age-related differences could not be attributed to an age-related alteration in tissue binding. The dialysis fraction of L-[$^{125}$I]$T_3$ in brain homogenates of young rats ($22.4 \pm 2.4\%$) was not different from that of the aged rats ($21.6 \pm 3.3\%$). Similarly, the dialysis fraction of L-[$^{125}$I]$T_3$ measured in the young rat plasma ($11.9 \pm 0.7\%$) was similar to the measurements in aged rat plasma ($11.9 \pm 1.4\%$). Table II summarizes the brain uptake index

TABLE II

THE BRAIN UPTAKE INDEX (BUI) OF L-$T_3$ AND D-$T_3$ IN YOUNG RATS, AGED RATS AND YOUNG RATS PAIR FED WITH AGED RATS (MEAN ±S.E.M.)
*$p<0.05$ compared to young rats.

| | *Young rats* ($n = 8$) | *Aged rats* ($n = 7$) | *Young rats pair fed with aged* ($n = 5$) |
|---|---|---|---|
| L-$T_3$ | $29.5 \pm 2.0$ | $22.4 \pm 2.1$* | $28.5 \pm 2.5$ |
| D-$T_3$ | $28.1 \pm 2.4$ | $23.2 \pm 1.8$ | $27.8 \pm 2.8$ |

(BUI) of L- and D-$T_3$ measured in young rats, aged rats, and young rats pair fed with aged rats.

The BUI of L-$T_3$ in aged rats ($22.4 \pm 2.1\%$) was significantly lower than that found in young rats ($29.5 \pm 2.0\%$) ($P<0.05$). The BUI of D-$T_3$ was also reduced in aged rats, but the difference did not achieve statistical significance. These changes could not be attributed to reduced food intake in aged rats since partial food restriction in young rats pair fed with aged rats did not alter the BUI of L- or D-$T_3$. The lack of significant differences in BUI of L-$T_3$ and D-$T_3$ is consistent with the notion that the carrier mediated transport of $T_3$ in rat BBB is only weakly stereospecific [17].

## DISCUSSION

These results clearly indicate that the BBB transport of $T_3$ is reduced in the aged rats. The reduction in BUI of $T_3$ with aging could not be attributed to a non-specific age-related change in BBB permeability nor to an age-related change in cerebral blood flow. We have previously found in the same strain of animals that the BBB uptake of tritiated water, the internal standard of $T_3$ BUI measurements, is not altered in aged rats [9]. In addition, the reduced cerebral blood flow in the aged rats found in the previous study [9], is expected to increase the $T_3$ BUI; and, therefore, the difference in the BUI found in old rats compared to the young rats would be further augmented.

Although the reduced food intake may contribute to the age-related reduction in $T_3$ transport to the liver [12], it does not appear to have an effect on BBB transport of $T_3$. Thus the L-$T_3$ and D-$T_3$ BUI's in young rats pair fed with aged rats were not different from young rats fed ad libitum. A variety of age-related changes in brain influx of nutrients have been reported [8]. Most of these studies, however, did not distinguish an age-related change in brain metabolism from an alteration in BBB transport function [2,18]. An age-related change in membrane fluidity [4,19] may contribute to the altered transport function. Alternatively, the number of $T_3$ carrier molecules may be reduced with aging. The molecular basis of altered BBB transport of $T_3$ with aging remains unknown.

It is noteworthy, however, that brain to plasma ratio of either L-$T_3$ or D-$T_3$ was not altered with age. This was not secondary to an age-related change in $T_3$ binding by plasma or the brain. It is likely, therefore, that the reduced BBB transport of $T_3$ with aging, coupled with reduced $T_3$ clearance is responsible for the lack of change in brain to plasma $T_3$ ratio. It is thus possible that the reduced BBB transport of $T_3$ is an adaptive response to the reduced $T_3$ metabolism in the aged brain. Such adaptive changes in rat BBB transport have been found for short-chain monocarboxylic acid carrier in response to fasting [3] and for glucose transporter in response to chronic hyperglycemia [7]. An age-related decline in $T_4$ to $T_3$ deiodination in the brain tissue has been recognized in

Long-Evans rats [6]. It is possible, therefore, that $T_3$ deiodination may well be reduced in the aged brain. Further studies are needed to determine whether the age-related reduction in BBB transport of $T_3$ is a primary defect of the BBB or is a secondary response to the reduced $T_3$ metabolism. An age-related change in the stereospecificity of BBB $T_3$ transport would have indicated that the reduced $T_3$ BUI observed in the aged rats is the result of a primary change in the BBB rather than an adaptive response to altered metabolism. However, there were no significant alterations in the ratio of brain uptake of L-$T_3$ to brain uptake of D-$T_3$ in aged rats compared to young controls.

At present, it is not clear whether the reduced BBB transport of $T_3$ with aging results in altered brain response to thyroid hormones. When brain-specific biomarkers of thyroid hormone action are identified, it would be possible to correlate BBB transport of $T_3$ with brain response to thyroid hormones.

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