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Subcellular Levels of L-T3 and L-T4 in Adult Rat Brain Cerebral Cortex

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Abstract. **Background.** Thyroid hormones exert major well-known functions in the growth and differentiation of most types of mammalian tissues. The hormones exert these effects via interaction with nuclear thyroid-hormone receptors, which regulate target gene expression. Thyroid dysfunctions in adult individuals have been linked to several neuropsychological disorders that can be corrected by appropriate adjustments of circulating thyroid hormones. However, the actions of thyroid hormones in the mature brain are unclear. As the mammalian brain approaches adulthood, thyroid hormone concentrations within nuclei decrease and in nerve terminals increase. These ontogenic differences and the changes in the subcellular localization and concentration of thyroid hormones are important in the exploration of thyroid hormone function in the adult mammalian brain. **Aim.** The aim of the present study was to compare the concentrations of L-triiodothyronine ($L\text{-}T_3$) and L-tetraiodothyronine ($L\text{-}T_4$) in two preparations from adult rat brain. **Procedure.** $L\text{-}T_3$ and $L\text{-}T_4$ concentrations were determined by radioimmunoassay. Comparisons were made of the concentrations of the hormones in two different preparations derived from young adult rat brain cerebral cortex: (1) purified synaptosomes (an artificial preparation of a subcellular fraction considered to have most biological properties of nerve terminals) and (2) non-synaptic mitochondria. **Results.** The concentration of $L\text{-}T_3$ was found to be ~3-fold higher in non-synaptic mitochondria compared to synaptosomal $L\text{-}T_3$ concentrations. The assay used did not detect $L\text{-}T_4$ in either fraction. **Conclusion.** The higher concentrations of $L\text{-}T_3$ compared to $L\text{-}T_4$ in the two different prepared fractions indicates a pivotal biological role of $L\text{-}T_3$ in adult mammalian brain.

Keywords • Cerebral cortex • $L\text{-}T_3$ • $L\text{-}T_4$ • Mitochondria • Ontogeny • Synaptosome

INTRODUCTION

Thyroid hormones have well-known vital functions in growth, differentiation, and development of almost all types of mammalian tissues including the brain. The classical mechanism of thyroid hormone action involves interactions with their specific nuclear receptors which in turn regulate target gene expression.[1] Through an offset of this mechanism, dysthyroidism adversely affects the developing brain, causing abnormal neuronal development that can lead to cretinism or myxedema in humans. Hypo- or hyperthyroidism, thus, leads to impairment of normal brain function in neonates. Thyroid disorders in adult humans have been shown to have several clinical neuropsychological, and behavioral adverse effects. Proper adjustment of circulatory thyroid hormone concentrations reduces or relieves these symptoms while the underlying neural mechanism remains unclear.

Immunocytochemical localization studies have shown that thyroid hormone receptors in adult vertebrates are highly concentrated within the choroid plexus, dentate gyrus, hippocampus, amygdaloid complex, pyriform cortex, granular layer of cerebellum, mammillary bodies, and the medial geniculate bodies. Although specific nuclear receptors for thyroid hormone in adult brain have been identified, their function in relation to target gene expression is not yet

completely clear. As the brain approaches adulthood, the nuclear iodothyronine level gradually declines and then reaches and maintains a plateau, and in adult vertebrates, thyroid hormone levels increase within nerve terminals.[2]

These switching differences in thyroid hormone ontogeny between developing and adult vertebrate brain has gradually come to interest investigators in their search for new functional roles and mechanisms of thyroid hormone action. Recently the concept of nongenomic actions of thyroid hormones has become popular.[3,4] The functional role of thyroid hormones in adult mammalian brain remains to be clearly understood.

Thyroid hormones are also concentrated and metabolized within nerve terminals of adult rat brain, particularly within synaptosomes, which are a artificial and nucleus-free sub-cellular fraction prepared from adult rat brain.[5-8] However, determination of the concentration of thyroid hormones in other subcellular compartments is needed. To help meet this need, the study reported here quantified and compared the levels of L-T_3 and L-T_4 in purified synaptosomes and non-synaptic mitochondria prepared from adult rat brain cerebral cortex.

METHODS

Animals. Three-month old adult male Charles Foster rats were maintained in a temperature-controlled room (24 ± 1°C) with a 12 h light-dark cycle, and fed ad libitum with a standard rat diet.

Preparation of synaptosomes and non-synaptic mitochondria. The animals were sacrificed quickly by decapitation. The brains were removed and the cerebral cortices were dissected out in an ice-cold condition. The synaptosomes from the cerebral cortex were prepared as described elsewhere.[9] Briefly, the cerebral cortex was homogenized (10 % w/v) in 0.32 M sucrose and centrifuged at 1000 X g for 10 minutes to remove cell debris and nuclei. The supernatant was collected and re-centrifuged at 1000 X g for another 10 minutes. The resulting supernatant was layered over 1.2 M sucrose and centrifuged at 34,000 X g for 50 minutes at 4°C. The fraction collected between the 0.32 M and 1.2 M sucrose layer was diluted at a 1:1.5 ratio with ice-cold bi-distilled water, further layered on 0.8 M sucrose, and again centrifuged at 34,000 X g for 30 minutes. The pellet thus obtained was washed and re-pelleted at 20,000 X g for 20 minutes. Intact synaptosomes were used for the experiments. The bottom pellet in the tube in 1.2 M sucrose was collected, suspended in 5 ml of 0.32 M sucrose and centrifuged at 10,000 X g for 20 minutes and. The washed pellet was used as non-synaptic mitochondria. For the assay of thyroid hormone concentrations, both of the subcellular fractions, the synaptosomes and non-synaptic mitochondria, were repelleted and ruptured hypo-osmotically by resuspending the pellets in ice-cold double-distilled water in ice for 30 minutes with occasional vortexing each 3 minutes.

Radioimmunoassay. Radioimmunoassay (RIA) of the total L-thyroxine (L-T_4) and total L-triiodothyronine (L-T_3) concentrations in serum, synaptosomes, and non-synaptic mitochondria were determined using an RIA kit supplied by Diagnostic Products Corporation, California, USA. All the samples were analyzed in triplicate in four separate experiments. The sensitivity of the L-T_4 and L-T_3 assay were 0.25 g/dL and 7 ng/dL, respectively, of the samples based on 90% B/B_0 intercept where B is the corrected average of standard/sample, and B_0 is the corrected average count of zero standard.

The L-T_4 assay kit showed only 2% cross reactivity with L-T_3 and triiodothyroacetic acid. The L-T_3 assay kit showed 0.38%, 1.1 %, and 0.014% cross reactivity with L-T_4, D-T_4, and reverse-T_3, respectively. Eighty-five to ninety percent of the hormones added to the ruptured subcellular fractions were recovered with the RIA kits. The RIA kit reagents contained 8-anilino naphthosulphonic acid that makes L-T_3 and L-T_4 free from the protein bound form. Hence, the chances for nonspecific binding of the hormone with the subcellular proteins were minimum. This is a direct procedure to measure the thyroid hormone concentrations within brain tissues without prior extraction of the hormones as followed in other conventional RIA methods.

Protein concentrations were measured by the method of Vera[10] using bovine serum albumin as the standard.

Statistical Analysis. Results were expressed as mean SEM of three separate observations. Each observation was made from 4 rats. Statistical analyses of the data were made by performing Student's t-test with $p < 0.05$ as the significance cut-off point.

Results

Comparison of the levels of L-T_4 and L-T_3 in subcellular fractions. Serum levels of L-T_4 and L-T_3 were normal. With this assay system, L-T_4 could not be detected in either synaptosomal or non-synaptic mitochondrial fractions. However, the L-T_3 concentration in synaptosomes and non-synaptic mitochon-

dria was appreciable. The concentration of L-T_3 in non-synaptic mitochondria was 3.2-fold higher than in the synaptosomal fraction purified from cerebral cortices (Table 1).

Table 1. Subcellular levels of thyroid hormones.

Hormones	Serum (ng/ml)	Synaptosome (ng/mg protein)	Non-synaptic Mitochondria (ng/mg protein)
L-T4	41 ± 0.2	ND*	ND*
L-T3	0.7 ± 0.03	0.45 ± 0.06	1.44 ± 0.12

*N.D. = Not Detectable

Discussion

Thyroid dysfunctions in adult humans are known to cause several neuropsychiatric diseases, some of which have been responsive to thyroid hormone therapy. For example, thyroid hormone deficiency has been linked to depressive illness, unipolar depression, affective disorders, and mental dysfunctions.[2]

Recent molecular mechanisms have demonstrated a crucial role of thyroid hormones in signal transduction in the adult brain. The signal transduction is mediated through protein phosphorylation/dephosphorylation.[11-13] The regulation of protein phosphorylation and dephosphorylation, including the involvement of second messenger molecules, have intense implications for thyroid dysfunctions in the adult mammalian central nervous system. Determination and distribution of brain tissue concentrations of thyroid hormones are thus important to understanding the mechanisms of thyroid hormone action in adult humans.

The present report quantifies the thyroid hormone concentrations from adult rat brain synaptosomes and non-synaptic mitochondria. Although L-T_4 levels could not be detected in synaptosomal and non-synaptic fractions, fair amounts of L-T_3 were detected in these fractions purified from adult rat brain cerebral cortex.[6] The finding of undetectable levels of synaptosomal L-T_4 is consistent with the finding in other studies.[7,14,15]

Type II iodothyronine 5'-deiodinase enzyme (5'-DII) is responsible for the conversion of L-T_4 to L-T_3 and appears to be more active in hypothyroidism. Despite low serum levels of thyroid hormone in hypothyroidism, L-T_3 production in the brain has been reported to be very high during stresses such as hypothyroidism.[6] 5'-DII has also been shown to be activated during other types of stresses, apparently playing a protective role in stressed brain.[16] Stimulated levels of 5'-DII has also been described during hypothyroidism, and this supports the initial report[6] of elevated brain L-T_3 concentrations during n-propylthiouracil-induced hypothyroidism.[7,8]

In the brain, approximately 80% of the L-T_3 is produced locally from L-T_4 by 5'-DII. The fractional rate of conversion of L-T_4 to L-T_3 is high in brain.[17,18] This might be a possible cause for undetectable L-T4 concentrations in the fractions used in this study. To detect endogenous thyroid hormones, the subcellular fractions were ruptured hypo-osmotically. The use of 8-anilinonaphtho-sulfonic acid in the RIA medium excluded the possibility of the non-detectable protein bound form of thyroid hormone by releasing the endogenously bound form of the hormones.

A comparison between the subcellular fractions tested showed that the L-T_3 levels in the non-synaptic mitochondrial fraction were ~3.2-fold higher than in the synaptosomal fractions. Levels of L-T_4 remained undetected. Comparatively higher levels of L-T_3 in the mitochondria may have implications for mitochondrial energetics, such as cellular oxygen consumption, oxidative phosphorylation, and ATP synthesis, which is one of the major regulatory functions of thyroid hormone.[19] Thyroid hormones have also been shown to affect the mitochondrial genome. Its effects are mediated by imported isoforms of nuclear thyroid hormone receptors that bind thyroid hormones, especially L-T_3 and L-T_2, and influence various mitochondrial transcription factors.[3]

The localization and concentration of radiolabeled L-T_3 within nerve terminals was the first landmark research described in adult rat brain.[20] This was followed by the immunohistochemical mapping that demonstrated that locus ceruleus norepinephrine stimulate the active conversion of L-T_4 to L-T_3. This established a morphologic co-localization of central thyronergic and noradrenergic systems.[5] Overall thyroid hormone levels within different compartments of the brain might be found to have discrete, differential, and potential regulatory functions for neurotransmission within adult mammalian brain.

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