Synaptosomal T_3 Content in Cerebral Cortex of Adult Rat in Different Thyroidal States

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The quantitation of thyroid hormone levels in cerebrocortical synaptosomes of the adult rat at altered thyroid states was studied because of the recent idea of the involvement of thyroid hormone in the adult mammalian brain. In contrast to a marked fall in serum T_3 levels in PTU-induced hypothyroid rats, the synaptosomal T_3 content was raised by 9.5 fold in this hypothyroid situation compared to the euthyroid control rats. A single injection of T_3 (2 μg/g) to the hypothyroid rats lowered the T_3 level to about half of those values obtained in only hypothyroid condition. The T_4 levels remained below the detectable range in all the groups tested. This may be because 70% to 80% of the intraneuronal T_3 comes through the local deiodination of T_4. The present investigation indicates a tendency for synaptosomal reorientation of T_3 level for vital neurophysiologic function in altered thyroid conditions. ***[Neuropsychopharmacology 11:151–155, 1994]***

KEY WORDS: *Rat brain; Cerebral cortex; Synaptosomes; Hypothyroid; L-Thyroxine; L-Triiodothyronine.*

Although high affinity, low capacity nuclear thyroid hormone receptors have been localized in the adult rat brain, no physiological parameter has been identified that may be accepted as an expression of the thyroid hormone–receptor interaction in this organ (Eberhardt et al. 1978; Schwartz and Oppenheimer 1978; Valcana and Timiras 1978; Dozin-Van Roye and De Nayer 1979; Oppenheimer et al. 1980; Gullo et al. 1987a,b). Recent evidences have shown selective uptake of thyroxine (T_4) and triiodothyronine (T_3), rapid conversion of T_4 to T_3, and specific ^{125}I-T_3-binding sites in synaptosomal fraction of nervous tissue in the adult rat brain. This favors direct nonnuclear action of thyroid hormone related to neurotransmission in the adult mammalian brain (Dratman et al. 1976; Dratman and Crutchfield 1978; Kastellakis and Valcana 1989; Hashimoto et al. 1991).

To further support that thyroid hormone is important in the normal neural function of the adult mammalian brain, thyroid hormone concentrations in synaptosome from the adult rat cerebral cortex in normal and different thyroid states have been determined.

MATERIALS AND METHODS

Animal Treatment

Adult male Charles Foster rats (180–200 g body weight [BW]) were maintained in a temperature controlled room (24 ± 1°C) with a 12 h light–dark cycle, and fed *ad libitum* with standard rat diet. Some of the rats were made hypothyroid by daily intraperitoneal (IP) injection of n-propylthiouracil (PTU) (Sigma Chemical Co., USA) at a dose of 2 mg/100 g BW for 14 days. Some of the hypothyroid and euthyroid control rats were injected IP (single) with T_3 (Sigma Chemical Co., USA) at a dose of 2 μg/g BW. Simultaneously, a group of euthyroid control rats were injected with the same volume of vehicle (alkaline 0.9% saline). All the rats were sacrificed after 24 hours of the last injection of T_3, or PTU, or PTU + T_3.

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Table 1. T_4 and T_3 Concentrations in Rat Serum

Group	T_4 (μg/dl)	T_3 (ng/ml)
Euthyroid	4.30 ± 0.20	0.69 ± 0.04
Hypothyroid	0.41 ± 0.03[a]	0.14 ± 0.02[a]

Results are expressed as mean ± SEM of 3 separate observations. Each observation was made from 6 rats.
[a] Significantly different from corresponding value, $p < .001$.

The serum samples were collected and stored at −70°C until use.

Preparation of Synaptosomes

The synaptosomes from the cerebral cortex were prepared according to the method of Sarkar and Ray (1992a). Briefly, the 10% cortical homogenate (in 0.32 M sucrose) was layered in 1.2 M sucrose, after removal of the cell debris and nuclei, and centrifuged at 34,000 ×g for 50 min at 4°C. The fraction collected between the 0.32 M sucrose layer and the 1.2 M sucrose layer was diluted at 1:1.5 with ice-cold, double-distilled water, further layered on 0.8 M sucrose, and again centrifuged at 34,000 ×g for 30 min. The pellet obtained was washed, repelleted at 20,000 ×g, for 20 min, and ruptured hypo-osmotically with 5 mM imidazole-HCl buffer at pH 7.4 at 4°C. The ruptured synaptosomal suspension was used for T_4 and T_3 radioimmunoassay (RIA).

Radioimmunoassay

RIA of T_4 and T_3 concentrations in both serum and synaptosomes were measured using RIA kits supplied by the Board of Radiation and Isotope Technology, Bombay, India. All the samples were analysed in duplicate. The sensitivity of the T_3 and T_4 assay was 0.24 ng/ml and 0.5 μg/dl, respectively, of the samples based on 90% B/Bo intercept where B is the corrected average counts of standard/sample, and Bo is the corrected average counts of zero standard. The T_4-assay kit showed 5% cross-reactivity and 0.1% cross-reactivity with T_3 and T_2, respectively. The T_3-assay kit demonstrated a cross-reactivity at 0.1% with both T_4 and T_2. Eighty percent to eighty-five percent of the hormones added to the ruptured synaptosomal suspension were recovered with the RIA kits.

Protein concentration was measured by the method of Vera (1988), using bovine serum albumin as standard.

Statistical Analysis

Results are expressed as mean ± SEM of three separate observations. Each observation was made from six rats. Statistical analysis of the data were made by performing Student's t test with $p < .05$ as the significance cutoff point. The multiple groups were also checked with Duncan Multiple Range Test.

RESULTS

Serum T_4 and T_3 Levels

An appreciable fall in the serum T_4 and T_3 levels was observed in hypothyroid animals compared to the control euthyroid values (Table 1).

Synaptosomal Concentrations of T_4 and T_3

In synaptosome, opposite nature of changes were noticed (Table 2). Hypothyroid rats showed a 9.5 fold higher ($p < .001$) amount of T_3 in the cerebrocortical synaptosomes compared to that found in euthyroid synaptosomes. A single injection of T_3 (2 μg/g) to the hypothyroid rats reduced (1.6 fold, $p < .001$) the synaptosomal T_3 level in comparison to the hypothyroid rats. An elevation (2.5 fold, $p < .001$) of the synaptosomal T_3 level was found in euthyroid control rats after a single injection of T_3 (2 μg/g).

The T_4 level remained undetectable in synaptosomes in all the experimental conditions in the present studies.

Table 2. T_3 Concentrations in Rat Cerebrocortical Synaptosomes in Different Thyroid States

T_3 (ng/mg protein)				
(A) Euthyroid Control	(B) Hypothyroid	(C) Hypothyroid + T_3 (2 μg/g)	(D) T_3 (2 μg/g)	F Values (one way ANOVA)
0.43 ± 0.06	4.10 ± 0.06	2.56 ± 0.14	1.07 ± 0.24	163.60 $p < 0.001$

T_3 results are expressed as mean ± SEM of 3 separate observations. Each observation was made from 6 rats. The values between the groups A-B, A-C, A-D, B-C, B-D, and C-D are statistically significant within $r < 0.05$ in Duncan Multiple Range Test.

DISCUSSION

Despite a number of recent evidences that indicated uptake and concentration of ^{125}I-T_3 in rat cerebral cortex synaptosomal fraction, and favored a possible role of the thyroid hormone associated with the process of neurotransmission (Heninger and Albright 1975; Dratman et al. 1976; Dratman and Crutchfield 1978; Kastellakis and Valcana 1989; Serrano-Lozano et al. 1991), direct quantitation of the hormone in synaptosomes in different thyroid states was lacking. In the present study, an attempt was made to demonstrate how the T_3 level in synaptosomes alters in response to different thyroidal condition.

Hypothyroid condition showed an appreciable decline in both serum T_4 and T_3 level in rats in a usual way as found by other investigators (Larsen and Frumess 1977; Leonard et al. 1981; Cooper et al. 1983). Although it has been shown earlier that in hypothyroid condition, the whole brain, or different regions of the brain, maintain similar levels of T_3 compared to the euthyroid control rats through increased activity of 5'-deiodinase type II (5'D-II), and corresponding high fractional rate of T_4 to T_3 conversion (Obregon et al. 1978; Vigoroux et al. 1979; Crantz and Larsen 1980; Kaplan and Yaskoski 1980; Leonard et al. 1981; Leonard et al. 1984; Morreale de Escobar et al. 1988; St Germain et al. 1988; Escobar del Rey 1989; Serrano-Lozano et al. 1991), insufficient evidence is available except for a few recent reports (Sarkar and Ray 1992b; Mason et al. 1993) to quantitate the synaptosomal concentration of thyroid hormone. Approximately 8-fold higher concentration of T_3 has been found in synaptosome by Mason et al. (1993) compared to the whole brain in euthyroid rats. Our present observation of approximately 9.5-fold higher T_3 content in synaptosome of hypothyroid rats compared to the euthyroid controls may be the result of a higher fractional rate of T_3 production by increased activity of 5'D-II, and a correspondingly higher selective uptake and concentration of T_3 molecules in the synaptosomes to cope up with the physiological need of thyroid hormone in this tissue at this condition (Crantz and Larsen 1980; Kaplan and Yaskoski 1980). In euthyroid rat brain, selective uptake of ^{125}I-T_3 and its concentration in synaptosomal compartment have been demonstrated (Dratman et al. 1976; Dratman and Crutchfield 1978). In addition, the use of hypothyroid animals only after 14 days of PTU treatment, where some adaptive mechanisms still unknown in nature, do not reach the equilibrium as compared to the animals kept in chronic hypothyroid condition for a much longer duration as used by other workers (Kaplan and Yaskoski 1980; Leonard et al. 1981; Morreale de Escobar et al. 1988). This may be another reason for maintaining a high level of synaptosomal T_3 in our hypothyroid rats. Expression of the data in different forms such as per g organ (brain) basis, or per mg compartmental (synaptosomal) protein basis, as presented in our experiment, also becomes an additive factor for discrepancies among different groups of workers regarding the quantitative aspects of T_3 or T_4 in the brain (Morreale de Escobar et al. 1988; Escobar del Rey et al. 1989; Mason et al. 1993).

The fall in T_3 concentration in synaptosome prepared from T_3-treated hypothyroid rat cerebral cortex may be the result of inhibition of 5'D-II activity after 24 hours of the T_3 administration, in the presence of the considerable amount of exogenous T_3. An inhibition in the activity of 5'D-II has been observed within 4 hours of T_3 treatment to the thyroidectomized rats (Leonard et al. 1981; Leonard et al. 1984). A rise in the synaptosomal T_3 level in the hypothyroid rats, and a fall in the same in the T_3-treated hypothyroid animals after 24 hours of T_3-treatment, also reflects the tendency for a compensatory regulatory mechanism of thyroid hormone metabolism in the adult rat brain in altered thyroid conditions, although the nature of the mechanism remains unknown. T_3-treated control rats have shown higher levels of synaptosomal T_3, compared to the control values. This may be a result of the extra T_3 transport influenced by a high dose of exogenously administered T_3 (2 μg/g) in a similar way as observed by Dratman et al. (1976, 1978), and Kastellakis and Valcana (1989).

In our experiment, the T_4 level in synaptosomes remained undetected in untreated control groups and other experimental rat groups. This may reflect a state of rapid conversion of T_4 to T_3 in the brain by 5'D-II enzyme (Dratman and Crutchfield 1978; Obregon et al. 1978; Vigoroux et al. 1979; Crantz and Larsen 1980; Kaplan and Yaskoski 1980). Other researchers (Dratman et al. 1976; Dratman and Crutchfield 1978) have already shown that after IV administration of radiolabeled T_4 and T_3, the hormone is concentrated as T_3 in a synaptosomal fraction of the whole rat brain, and T_4 to T_3 conversion occurs very rapidly within the nerve cells. T_3 formed in the neuronal cell body then may be translocated down the axon to the synaptic ends. Kastellakis and Valcana (1989) also demonstrated a saturable and nonsaturable uptake of T_3 and T_4, respectively, in isolated synaptosomes in an in vitro model indicative of a two-component T_3-uptake system.

The procedure we developed for the direct radioimmunoassay of T_3 and T_4 in synaptosomes using a kit also appears as an alternative method for the thyroid hormone assay without prior extraction of the hormone as followed in other conventional RIA methods. As the RIA kit reagents also contained 8-anilino-naphthosulphonic acid that makes T_3 and T_4 free from the protein bound form, the chances for nonspecific binding of the hormone with synaptosomal protein materials are minimum. In addition, the data regarding cross-reactivity,

sensitivity, and recovery in our RIA system may support it to be a viable alternate, direct assay method.

The data emerged from our study reveal the quantitative aspects of involvement of T_3 in synaptosomes in different thyroid states, and favors its role in neuronal functions as formerly described (Dratman et al. 1976, 1978; Hashimoto et al. 1991). A stimulation of synthesis of synapsin-I protein (related to neurotransmission) by T_3 in the developing brain has been reported (Salemi et al. 1990). Although, the synaptosomal T_3 levels varied widely with different treatments, our results illustrate a unique, but unknown regulatory mechanism of the thyroid hormone metabolism in the mature mammalian brain.

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