In quest of thyroid hormone function in mature mammalian brain

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Thyroid hormones (TH) have important functions in maturation, differentiation and metabolism during developmental periods in almost all types of tissues including brain of vertebrate animals. In humans' thyroid malfunction in early developmental stages cause severe neuropsychological abnormalities due to defective gene expression via nuclear receptor activation. However, role of TH in adult mammalian brain is lacking and unclear mainly because it was considered for a long time as a TH unresponsive tissue. Although adult brain contains a substantial number of TH nuclear receptors, no functional properties could be attributed. Recent findings suggest that T3 is distributed, concentrated, metabolized and binds to specific membrane sites within adult brain. In mature humans TH also reversibly regulates various neuropsychological symptoms produced in mature condition. This review discusses development of recent concepts and literature on role of TH and its importance in neuronal function in adult mammalian brain.

Thyroid hormones gained importance in developing brain

Influence of TH in developing mammalian nervous system is dramatic. Use of thyroid extract therapy first by Murray to give a relief to hypothyroidism has been the premier footprint of mankind to enter into the therapeutic world of TH research. This was a great achievement for mankind as described by Sir William Osler: "We rescue children otherwise doomed to helpless idiocy is a triumph of experimental medicine. Within six weeks, a poor feebleminded toad like caricature of humanity may be restored to mental and bodily health". Thus the crucial role of TH for regulation of normal development and functioning of CNS gradually drew attention of the investigators. The most stunting observation is the maturation of the CNS. Thyroid dysfunction during early developmental stages causes several structural and functional abnormalities that lead to various neurological and psychobehavioral syndromes. Such as cretinism and associated neurological impairments provide important clinical reference to the impact of hypothyroidism on developing brain. All these effects of TH are mediated through the activation of nuclear T3-receptors.
Why look back to adult brain, once ignored tissue to search for thyroid hormone action?

Although the effect of TH on developing brain has been well documented, the role of TH in normal functioning of the adult mammalian brain is lacking and very much unclear, mainly because of a long-time notion that adult brain is unresponsive or has very low sensitivity to this hormone. It has been reported that adult brain is unable to increase the rate of oxygen consumption or calorigenesis followed by hormonal treatment\textsuperscript{8,9}. Although a substantial number of TH-nuclear receptors with an inability to bind T3 have been demonstrated in adult brain, no functional properties could so far be attributed\textsuperscript{3-10}. Obviously the question comes, "why search for TH action in adult brain?" The seriousness of conducting a search for TH action in mature neuronal tissues originates from the fact that adult persons develop a number of neurological and psychological changes during alterations in thyroid states. Most importantly these neurological and psychological symptoms developed in mature conditions could be corrected by proper adjustment of the circulatory TH. The present review is intent to particularly focus on the recent development of the idea of involvement of TH in adult mammalian brain.

Emerging evidences for involvement of thyroid hormones in adult brain function

Thyroid hormone nuclear receptors

\textit{Nuclear receptor isoforms}—T3 action is mediated by two nuclear receptor (TR) gene products, TRα and TRβ, formed by alternative splicing of α- and β-\textit{c-erbA} gene products respectively. The human TRα gene, located on chromosome 17, produces three different mRNA products, TRα1, TRα2 and TRα3. However, TRα1 is able to bind T3, whereas, TRα2 is not\textsuperscript{11}. The human TRβ gene located on chromosome 3, produces TRβ1 and TRβ2. Functional proteins encoded by both TRα and TRβ have similar biochemical and physiological properties. Both TRα and TRβ genes are coexpressed in some tissues\textsuperscript{3}.

\textbf{Expression of TRα and TRβ genes}—TRα2 mRNAs increase until postnatal day 7 and decrease thereafter until adulthood. TRα1 mRNAs increase between 16\textsuperscript{th} day and birth and remain unchanged until adulthood. TRβ1 mRNAs increase 3-fold between 15-16\textsuperscript{th} fetal day and postnatal day 13 where they attain their adult values. Although TRα2 has been described as an inactive form of the receptor by its non-bonding ability with T3, a transfection study suggests its role as blocker of T3 effect mediated by TRα1 and TRβ1 (Ref. 12). Thus information provided surmise that the apparent non-responsiveness of adult brain to T3 may be due to high levels of TRα2 isoform.

\textbf{Cellular and regional localization of nuclear T3 receptors}—Immunocytochemical studies have localized nuclear TR in astrocytes, neurons, and glial cells\textsuperscript{13,14}. Maximum TR immunoreactivity is located in the olfactory bulb, hippocampus, dentate gyrus, amygdala and neocortex (layer III-IV) of adult animals, with intermediate immunoreactivity in hypothalamus, whereas, thalamus, central gray matter, substantia nigra, interpedencular nuclei and striatum are shown to have minimum response. Nuclei of the Perkinje cells in cerebellum show a strong immunoreactivity that is also noticed within the internal granular layer and in some nuclei of cells located in the molecular layer\textsuperscript{14}. Although cerebellum is highly sensitive to stain by TR antibody, investigations have demonstrated the presence of an inactive form of TR in majority in adult rat brain cerebellum with an inability to bind T3 (Ref. 14). These may be the possible explanation to apparent inconsistency between presence of TR immunoreactivity and low level of T3 binding sites in adult brain cell nucleus. Use of highly sensitive double-label \textit{in situ} hybridization technique shows that estrogen receptor (ER) positive neurons expressing ERα also express TRα1 and TRα2 mRNAs in different brain regions of overectomized adult female rat including ventromedial and arcuate nuclei of the hypothalamus and amygdala\textsuperscript{15}. Immunocytochemical localization of TR in adult brain also confirms regional distribution of [\textsuperscript{125}I]-T3 after intravenous administration of [\textsuperscript{125}I]-T3, which is highly concentrated in choroid plexus, dentate gyrus, hippocampus, amygdaloid complex, pyriform cortex, granular layer of cerebellum, mammillary bodies and medial geniculate bodies\textsuperscript{16-18}. As the brain approaches adulthood, nuclear iodothyronine concentration declines gradually reaching a plateau and maintains it, and hormone concentration increases within nerve terminals of adult brain\textsuperscript{19}. Immunohistochemical mapping further suggests that locus coeruleus norepinephrine stimulates active conversion of T4 to T3. It also establishes a morphologic connection between central thyronegenic and noradrenergic systems\textsuperscript{20}. This change in TH ontogeny gradually started drawing attention that possibly TH action in mature brain
switches its role which may be different from its classical action through nuclear TR.

T3-binding to brain thyroid hormone nuclear receptors—Decrease in T3-binding has been shown in adult rat brain compared to developing brain. Hyperthyroidism causes an increase in maximum binding capacity (MBC) and affinity constant (Kd) of adult rat brain nuclear TR. A nuclear protein encoded by α1 subtype mRNA has shown a high affinity T3-binding capacity in cerebral cortex compared to cerebellum in adult euthyroid rats and these nuclear proteins possess analog specificity. The protein encoded by α2-mRNAs lacks the binding capacity to T3 and are thus receptor variants or non-T3-binding isotype. Reason for high receptor level in both hypo- and hyperthyroidism as observed, is explained to be due to existence of a functional heterogeneity in mature brain. An attempt to reproduce these data failed.

Thyroid hormone membrane receptors
Specific high affinity, low capacity T3-binding sites has also been described in synaptosomes and in synaptic membrane of adult rat brain with higher concentrations in hypothalamus and cerebellum of rat compared to cerebral cortex, and in mouse neuroblastoma plasma membrane. However, no physiological function(s) due to interaction of TH and its receptors has been documented so far in adult brain. We also have confirmed the existence of two types of specific neuronal membrane receptors for TH in adult rat cerebral cortex and have tested its specificity using different TH analogues, including T3-amine (T3-amine EDso = 10 nM, T3 EDso = 1 nM), a compound, yet not identified within the physiological system, but thought to be an amine derivative of TH having potential neurotransmitter-like activity, like other classical catecholamine neurotransmitters in brain. Previous studies have shown T3-binding sites in nerve terminals and in astrocytes from developing brain. Identification of specific T3-binding sites in rat thymocyte membrane, depolymerization of actin filaments in cultured astrocytes by TH, and changes in second messenger components after TH treatment.

Selective uptake of thyroid hormones by nerve terminals
Intravenous administration of [125I]-T4 in rats followed by thaw mount autoradiography also shows that T4 is distributed in selective areas of adult brain in a saturable manner and gradually is concentrated more within nerve terminals fractions, where T4 is monodeiodinated to produce T3, the active form of TH. T4 and T3 transportation within neurons are shown to occur by two different mechanisms. T3 is actively taken up in a saturable manner, while T4 transportation occurs by diffusion and in a non-saturable way. T4-transporation within neuron is dependent upon T4-concentration gradient between extracellular and intracellular compartments and is maintained by high deiodination rate of T4 to T3.

Thyroid hormone metabolism
Three important enzymes, generally called monodeiodinases and involved in TH metabolism, remove iodine atoms either from phenolic (5'-) or tyrosyl (5-) ring of iodothyronines. These are 5'-deiodinase type I (5'D-I), 5'-deiodinase type II (5'D-II) and 5-deiodinase type III (5'D-III). 5'D-II catalyzes the deiodination of T4 to produce T3, which is then further deiodinated by 5'D-III to inactive compound 3',5'-T2. In brain 5'D-I is predominantly found in glial cells and 5'D-II in neuronal plasma membrane of cerebral cortex, in neuroblastoma cells and in astrocytes from developing brain. 5'D-III may be present in both neurons and glial cells.

Despite much controversy, 5'D-II is of recent interest because it is thought to be involved in the conversion of T4 to T3 especially within nerve terminals. Interest originates to search for TH concentration within adult brain neuron, because of numerous supportive evidences that describe active involvement of TH in adult brain. Knowledge of mechanism regulating regional brain TH concentrations in adult brain is lacking.
Although, concentrations of TH in rat whole brain are in low nanomolar range\(^4\), T3 concentration within cerebrocortical synaptosomes prepared from adult rat brain has been shown to be approximately 14.6 nM after extracting hormones from synaptosomes.\(^{48}\) Almost simultaneously, we have also reported T3 concentration within cerebrocortical synaptosomes to be 0.43 ng/mg synaptosomal protein, equivalent to 13 nM (Ref. 52) in euthyroid rats, by a direct assay method using 8-anilinonaphthosulfonic acid to release endogenously bound hormones.\(^{51}\) Surprisingly, our assay condition shows approximately 9.5-fold higher T3 level (2.56 ng/mg protein; approx. 126 nM T3)\(^{55}\) in n-propylthiouracil (PTU)-induced hypothyroid synaptosomes, despite a 5-fold lower serum T3 levels compared to euthyroid values. We failed to detect any T4 level within synaptosomes from both euthyroid and hypothryoid cerebral cortex. We attribute this as a homeostatic mechanism during hypothyroidism, which compensate for lower circulating levels of TH\(^{50}\).

Despite low serum levels of TH, hypothyroid condition in whole brain or in different brain regions maintains similar levels of T3 compared to euthyroid rats through increased activity of 5’D-II, and corresponding high fractional rate of T4 to T3 conversion\(^{55-58}\).

In brain, approximately 80% of T3 is produced locally from T4 by 5’D-II\(^{53,59}\). 5’D-II plays a crucial role in protection of brain regions, and preservation of brain T3 levels, particularly in glial cells and interneurons of cortex against hypothyroidism\(^{57,58}\). Inhibition of 5’D-II activity observed within 4 hr of T3 treatment to thyroidectomized rats reflects a compensatory regulatory mechanism\(^{60}\). Furthermore, our observation, rise in T3 level in a stress situation, like hypothyroidism, is supported which has shown that even mild and brief stress could induce marked rise in T3 content in different brain areas of adult rat, whereas changes are not reflected in liver or blood in adult animals\(^{56,58}\). Activation of 5’D-II is also dependent on cAMP levels\(^{61}\). Thus, (i) increased activity of 5’D-II to produce more T3 locally, (ii) decrease in activity of 5D-III to reduce further metabolism of T3 to T2 or other inactive metabolites of TH, and (iii) unaltered activity of 5’D-I, have been described as a protective mechanism particularly in adult brain\(^{56,58}\). Although, physiological significance of 5’D-I in adult CNS is very much unclear, demanding necessity of T3 for providing a protective mechanism to adult brain is interesting.

Possible metabotropic role of thyroid hormones in neurotransmission

**Effect on Ca\(^{2+}\)-influx**—Demonstration of sequestration of TH within nerve terminal\(^{28}\) was the first footprint to search for a possible role of TH in neurotransmission\(^{20}\). One of the features for neurotransmitter is neurotransmitter release followed by Ca\(^{2+}\)-induced depolarization through voltage-gated ion channels.\(^{52-63}\) T3 has been reported to stimulate Ca\(^{2+}\)-influx by cerebrocortical prisms of hypothryoid mice.\(^{65}\) T3 also induces \(^{45}Ca\)-uptake in depolarized cerebrocortical synaptosomes in adult rats. Although, insignificant T3-induced \(^{45}Ca\)-uptake is noticed between euthyroid and hypothyroid rats, no physiological relevance for this has been discussed\(^{51}\). Later on using quin-2 as a fluorescence probe we have described a dose-dependent T3-induced Ca\(^{2+}\)-influx in depolarized synaptosomes from both euthyroid and hypothyroid adult rat cerebral cortex, with a significantly higher rate (p<0.05) in hypothyroid animals\(^{56,60}\). We suggest an adaptive mechanism under condition of PTU-induced hypothyroidism, which elevated (9.5-fold) synaptosomal T3 concentration well above brain physiological concentration of T3 (Ref. 50). This enhancement of Ca\(^{2+}\)-influx, using fura-2 AM as fluorescence probe, is further confirmed in euthyroid synaptosomes and has been correlated with the rise in nitric oxide synthase activity indicating a definite non-genomic action of T3 in mature brain\(^{68}\).

**Effect on neuronal membrane enzymes**—TH is well known for its regulation of energy metabolism in developing tissues including brain. However, in adult brain it has not been described until recently. Maintenance of ionic gradients by plasma membrane Na\(^+\)-K\(^+\)-ATPase and Mg\(^2+\)-ATPase is one of the cellular processes by which TH regulate energy metabolism. Although, involvement of Na\(^+\)-K\(^+\)-ATPase has been described in different tissues of lower adult vertebrates\(^{69,70}\), only a few reports are available in adult mammalian brain\(^{71,72}\), which may be attributed for the pharmacological or toxicological effects of TH. Na\(^+\)-K\(^+\)-pump is important for maintenance and restoration of cation gradients that are needed for impulse propagation and for general cation homeostasis in the nerve tissues. Especially in neurons it regulates neurotransmitter release\(^{71}\). We have demonstrated that T3 inhibited the synaptosomal Na\(^+\)-K\(^+\)-ATPase activity in a dose-dependent manner in vivo\(^{49}\) and in vitro\(^{72}\). Furthermore, we have correlated this inhibition of
enzyme activity with gradual binding of $[^{125}\text{I}]-\text{T3}$ to specific T3-binding sites in synaptosomes. This report illustrates a physiological response followed by binding of T3 to its membrane binding sites $^{78}$.

We have also shown that T3 stimulates acetylcholine (Ach) metabolism in adult rat brain cerebral cortex by increasing acetylcholinesterase (AchE) activity and the uptake of Ach by stimulating synaptosomal ouabain-insensitive $\text{Mg}^{2+}$-ATPase activity in both euthyroid and hypothyroid rats $^{51}$. An increase in immobility response in thyroidectomized rats is reported $^{73}$. Contrarily, an attenuation of immobile response after chronic PTU-treatment for 21 days also has been noted $^{75}$. Only chronic and high doses of T4 normalize or prevent increase in immobile response $^{74}$. An increase in AchE activity in young adult rat brain (3 months old) is predicted due to increase in membrane sphingomyelin content $^{76}$. In contrast, a significant reduction in AchE activity seen in subcortex of partially thyroidectomized adult rat (6 months old), may be attributed to difference in age and type of creation of hypothyroidism $^{77}$.

Membrane effects of thyroid hormones—Although high affinity synaptosomal T3 binding sites $^{56,28}$, and a correlation between gradual T3-binding and inhibition of synaptosomal Na$^+$/K$^+$-ATPase $^{78}$ activity have been mentioned, the receptor functions are yet to be established.

Submicromolar concentrations of T4 and T3 stereospecifically stimulate $[^{35}\text{S}].\text{t-butyl-bicyclophosphorothionate}$ binding (a convulsant ligand for GABA$\_\alpha$ receptor complex) in highly washed adult rat brain membranes. Higher concentrations of hormones inhibit radio-ligand binding indicating a bi-phasic effect of hormones. T3 also inhibits GABA-stimulated $^{36}\text{Cl}^-$ flux in synaptoneurosomes of adult rat brain cerebral cortex, as well as in recombinant GABA$\_\alpha$ receptors expressed in human embryonic kidney-293 cells $^{52}$. This short-term effect further clearly shows another evidence of non-genomic action of TH in adult brain.

Other effects of thyroid hormones in adult CNS—Recently TH have been shown to influence a few gene expression in adult brain, like expression of hypothalamic peptide mRNA $^{78}$, nerve growth factor (NGF), neurotrophin-3 (NT-3), brain derived neurotropic factor (BDNF) mRNA $^{79}$, and RC3/neurogranin mRNAs (which encodes for neuronal dendritic protein) in discrete brain areas $^{57,80}$.

Administration of T3 to hypothyroid animals could not revert mRNA levels of 5'D-II gene to normal levels. Thus direct effect of T3 on 5'D-II gene is disregarded, whereas increase in 5'D-II activity may be an indirect and distal to the primary effect of hormone $^{57}$. Chronic T3 treatment downregulates cortical 5-HT$\_2A$ receptor (serotonin receptor subtype), which is further potentiated by chronic imipramine (anti-depressant) treatment in adult rat brain $^{81}$. Chronic T3 administration also stimulates 5-HT$\_1A$ receptors and induces downregulation of BDNF mRNAs expression in dentate gyrus region of hippocampus from adult rat brain $^{82}$.

Clinical features of thyroid dysfunction

Neuropsychological effects—TH dysfunction during early developmental stages leading to several structural and functional disorders are dramatic and well established $^{6}$, whereas the literature in adult brain are limited and inclusive. The clinical symptoms of adult-onset dysthyroidism strongly indicate impairment of important brain functions. Long-prevailed idea of unresponsiveness of TH to adult brain is strongly challenged by the observations of these clinical symptoms, which are clearly coupled to TH status.

In general, hypothyroidism is found in association with changes in certain psychotic behavior, delusions, hallucinations, ataxia, loss of $\alpha$-rhythm on electroencephalography (EEG), somnolence, and progressive intellectual deteriorations $^{87,83}$. In contrast, manifestations of hyperthyroidism are linked with nervousness, irritability, tremulousness, delirium, stupor, increase in the frequency of $\alpha$-rhythm, anxiety, emotional liability, and in some serious cases seizures and even coma $^{77}$.

Interactions have also been established between TH and depressive illness $^{84}$. A hypothalamo-hypophysio-thyroid disturbance associated with low T3 syndrome and reduced thyroid stimulating hormone (TSH) response for the release of thyrotropin releasing hormone (TRH) in humans in some primary depressive states has been observed $^{85}$. TH also exerts antidepressant effects in human subjects especially in patients who fail to respond to tricyclic antidepressant therapy $^{84,86}$. Patients with major manic depression and schizophrenia often show a significant decrease in total T4, free T4 and rT3 $^{87,88}$. Thyroid dysfunctions also lead to produce several types of depressive disorders $^{89}$, like unipolar depression $^{90,91}$, affective disorders $^{88}$, and mental dysfunctions $^{84}$. 
TH-treatment improves memory skills and some psychobehavioral symptoms\(^2\), regulates cognitive functions and mood disorders\(^2\). Some studies predict a potential role of cholinergic mechanism during dysthyroidism\(^{51,94}\), which may be considered as possible risk factor for senile dementia of Alzheimer type (SDAT), since hypothalamic hypothyroidism caused cognitive disability\(^{95}\).

Role of thyroid axis activity and serotonin function in major depressive episodes has also been discussed, which predicted a defective TSH response and serotonin dysregulation\(^9\). TH-mediated modulation of activation of serotonergic neurotransmission via stimulation of 5-HT\(_2\) receptor and reduction in 5-HT\(_{1A}\) autoreceptor, in affective illness, is also suggested\(^{97}\).

TH also significantly regulates sleep disorders in mature conditions. A microinjection of T3 to medial preoptic area significant increases REM sleep, whereas microinjection of T3 in median preoptic nucleus significantly inhibits non-REM sleep. This suggests a non-genomic function of TH in adult brain\(^9\).

Although the quest for the role of TH is not yet over, in near future further investigations will reveal the potential function of thyroid hormones in adult brain in the fascinating world of diverse human psychobehavioral interactions and higher mental functions.

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