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The effect of triiodothyronine on the hippocampal long-term potentiation in an animal model of the Alzheimer's disease: The role of BDNF and reelin

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ABSTRACT

Converging evidence, propose a close relation between thyroid function and Alzheimer's disease (AD). We have assessed the effect of subcutaneous and intrahippocampal administrations of triiodothyronine (T3) on the electrophysiological activity (hippocampal long-term potentiation (LTP)), the levels of thyroid hormones (THs) and TSH, the protein expression of BDNF and reelin as well as histological changes in the hippocampus of AD rats. Beta-amyloid (Aβ) plus ibotenic acid (Ibo) were injected intrahippocampally and rats were treated with T3 or saline. The hippocampal levels of THs and the protein expression are measured by ELISA kits and Western blotting method respectively. Results have been shown that T3 (S.C., and I. H), significantly reversed the amplitude and the slope impairment of the DG neurons, induced by Aβ. The hippocampal levels of THs, TSH and two protein expression were significantly decreased ($p < 0.001$) in AD animals and increased significantly in AD rats that have received T3 (S. C and I. H) ($p < 0.01$). Formation of amyloid plaques was declined in AD rats treated with T3. In conclusion, both S.C., and I.H. injections of T3 is effective in preventing the disruption of synaptic plasticity induced by Aβ. This positive effect of T3 may be mediated through a regulation of proteins expression and the hippocampal level of THs. The best effect was observed in I.H. microinjection of T3.

1. Introductions

Alzheimer's disease (AD), a progressive damages of neurons that, accompanied by neuropsychiatric disorders, such as unusual behaviors, personality changes and a decline in thinking abilities (Robins Wahlin & Byrne, 2011; Mirakhur, Craig, Hart, Mc Llroy, & Passmore, 2004). Extracellular amyloid beta (Aβ) plaques are the one of two main hallmarks of AD (Henry, Querfurth, & La Ferla, 2010). Aβ peptide was created by proteolysis of the amyloid precursor protein (APP) and it is a crucial trigger for disruptive effects on neurons and oxidative injury in AD (Kelly & Ferreira, 2006). One of the possible causes for Aβ deposition is a damaged clearance of Aβ at the blood-brain barrier (BBB) and the oxidative stress probably plays an important role in this process (Ehrlich, Hochstrasser, & Humpel, 2013). Our study newly revealed that intrahippocampal injection of Aβ decreased different types of memory, such as passive avoidance and spatial memories (Shabani et al., 2016; Farbood et al., 2017). Different clinical and laboratory reports indicated that body weights decreased in AD patients and Aβ induced AD rats. Although, the exact reasons for weight loss are unclear, but this may have depended on stage and severity in AD diseases (Intebi et al., 2003). There are evidences about the involvement of brain-derived neurotrophic factor (BDNF), a member of the neurotrophin superfamily, and reelin, an extracellular matrix protein, in AD pathogenesis (Mattson, Maudsley, & Martin, 2004; Pujadas et al., 2014). Reelin and BDNF are expressed in the hippocampus and cortex (Murer, Yan, & Raisman-Vozari, 2001). Theses play a very vital role in neuronal maturation and survival, differentiation, and synaptic plasticity in the brain (Hethorn et al., 2015; Mattson et al., 2004). Previous finding showed a decline of reelin and BDNF protein expression in the hippocampus of AD mouse (Shabani et al., 2017; Chin et al., 2007). Aβ peptide has been reprted to the reduction BDNF expression (Rosa & Fahnestock, 2015). Furthermore, the reduction of reelin protein expression results in advanced formation and age-related aggregation of Aβ plaques (Kocherhans et al., 2010). Two types of thyroid hormones (THs) including triiodothyronine (T3) and thyroxine (T4) are essential regulators of development and differentiation of the neurons in the central nervous system (Stenzel & Huttner, 2013). The reduction of expression APP (a precursor of Aβ) in the brain by THs may propose a

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very close association between thyroid dysfunction and AD pathology (Contreras-Jurado & Pascual, 2012; Davis et al., 2008). HPT axis abnormalities have identified in clinical reports of AD patients. Individuals with lower TSH levels (outside of the standard range) revealed a greater than threefold enhanced risk of dementia (Kalmijn et al., 2000). The pervious evidences showed that the prefrontal cortex of brain patients with clinical features of AD might be in a state of tissue hypothyroidism (Davis et al., 2008). In addition, a decrease in level of T4 and T3 within the standard range is a strong predictor of cognitive impairment in women (Volpato et al., 2002). Additionally, the reduction of BDNF and reelin expression has been seen in the hypothyroidism rat cortex and hippocampus (Sui & Li, 2010; Alvarez-Dolado et al., 1999). In the current investigation, we aimed to evaluate the protective effect of S.C and I.H injections of T3 on Aβ-induced histological changes, alterations in expression of BDNF and reelin and evaluation of hippocampal THs and TSH Levels in AD rats.

2. Material and methods

2.1. Preparation of triidothyronin (T3)

Triiodothyronin Powder was dissolved in 0.1 M NaOH (PH = 7.4) and diluted with different serum volume in order to reach a final concentration of 25 μg/kg and 50 pmol/μl (Shabani & Mirshekar, 2018; Wu, Liu, Wang, & Ruan, 2011). The S.C. injection of T3 was done into the skin behind the neck.

2.2. Preparation of beta-amyloid peptide 1–42 (Aβ1–42)

Aβ powders dissolved in 0.01 M PBS (PH = 7.4) at the concentration of 10 ng/μl. With the aim of forming the aggregated Aβ, the acquired solution was incubated at 37 °C (5 days) and slowly and bilaterally injected into the DG (Ogino et al., 2014; Shabani et al., 2016).

2.3. Preparation of ibotenic acid (Ibo)

Ibo powders (0.5 μl at 0.6 μg/μl) was also dissolved 0.01 M PBS (PH = 7.4) and 48 h after Aβ infusion, injected into the DG region of hippocampus bilaterally (Ogino et al., 2014).

2.4. Animals

Forty five male Wistar rats (250–300 g) were provided by the animal house of the Ahvaz Jundishapur University of Medical Sciences and equally divided into four groups, including: 1) Sham operated + Veh (Sh-O), 2) The AD groups received Aβ (10 ng/μl) and ibotenic acid (0.6 μg/μl, 48 h after Aβ infusion) at the DG region, and after one week received 0.2 μl normal serum, 3–4) The AD + T3 groups received 50 pmol/μl and 25 μg/kg doses of T3 (I.H and S.C, for ten consecutive days respectively). Animals kept at standard condition (temperature 22 ± 2 °C, 12 h light/dark cycles, free access to food and water). All animal experiments were totally done in accordance with the principles set by the Local ethics committee (Ethics Code: IR.AJUMS.REC145).

During intrahippocampal infusion of normal saline as vehicle (triidothyronine solvent) in AD + veh (I. H) group did not observe any significant difference compared with S. C injection of vehicle (normal serum) in AD + veh (S.C.) group. Hence, with the aim of clearly data presentation, we used here just one of the AD + veh groups.

2.5. Aβ and Ibo injections

Each anesthetized rats (ketamine/xylazine (90/10 mg.kg^{-1})) were placed in a stereotaxic apparatus (Narishige Co, Tokyo, Japan) and the Aβ (3 μl/side) and Ibo (48 h following Aβ injection, 0.5 μl/side) were slowly and bilaterally microinjected (via 30-gauge needle coupled to a 10 μl Hamilton microsyringe) into the DG area of the hippocampus (AP = −3.8 mm, ML = ± 3.5 mm, DV = −4 mm, according to the Paxinos and Watson atlas (Paxinos & Waston, 2006). The surgical animals were given a five days recovery between ibotenic acid administration and the starting S.C injection of L-T4.

2.6. LTP recording

Briefly, the bipolar metal wire recording (tungsten wire, CFW Co., USA) and stimulating (stainless steel wire, CFW Co., USA) microelectrodes were imbedded into the DG area (AP = ± 3.8 mm, ML = ± 3.2 mm, DV = 2.7 mm) and in the perforant pathway (PP) (AP = -7.5 mm,ML = −4 mm, DV = 3.9 mm) respectively. In the first step, single monopolar pulses (duration 50 μs, at 30 s intervals) delivered to the PP and field excitatory postsynaptic potentials (fEPSPs) recorded from DG area. In the second step, the fEPSP and high-frequency stimulation (HFS) with 40% and 80% of its maximum amplitude were carefully selected as baseline intensities and tetanic stimulation by an input/output (I/O) curve, respectively. The produced extracellular field potential was amplified (×1000), filtered (0.1Hz-3 kHz), digitized (2 kHz) and stored on the computer(Science Beam Co. Version 1.107, Iran). In the third step, to induce LTP, HFS protocol (6 trains of 6 pulses (50 μs) at 400 Hz, 100 ms intervals between each train) was used and measured Amp and fEPSP slope. The input/output responses and field potential recordings were found following stimulation of the PP in granular cells of the hippocampal DG.

2.7. Assessment of thyroid hormone levels

After deep anaesthetize, the brains of animals were rapidly removed, frozen in liquid nitrogen, weighted and homogenized in buffer phosphate (0.1 M BF, PH = 7.4). Following homogenization (500 mg tissue/ 1 ml of BF), the samples were agitated in a shaker and then centrifuged (12,000 rpm, 10 min). The homogenate supernatants were collected and THs and TSH levels were determined in brain samples of all animals. The concentrations of T_4, T_3, FT_4I, FT_3I and TSH in homogenate supernatants were measured by ELISA assays kits (Monobind USA Inc). The minimum detectable dose of TSH is < 0.027 μIU/ml and the sensitivities for T_4 and T_3 were 0.128 μg/dl and 0.04 ng/ml, respectively. Also, FT4I and FT3I values were carefully calculated.

hippocampus tissue were separated of the brain other sections, then 100 mg of frozen hippocampus tissue extracted by a radio immunoprecipitation assay (RIPA). The extracted proteins of tissue were resuspended in (1%) SDS to analyze the protein fraction. The concentration hippocampus proteins were carefully determined by Bradford's assay.

2.8. Western blotting analysis

Separated hippocampus proteins by SDS-PAGE on acrylamide gels (10%), were gradually transferred onto a nitrocellulose membrane and blocked using non-fat dry milk (5%) dissolved in Tris-buffered saline with 0.1% Tween 20 (TBST, pH 7.6) for 8 h. Then, gradually incubated overnight at 4 °C with anti-BDNF (dilution 1:1000; rabbit polyclonal, Abcam [ab205067]; USA), anti-reelin (dilution 1:1000, mouse monoclonal, Abcam [ab78540]; USA) and anti-beta Actin antibodies (dilution 1:5000, mouse monoclonal; Abcam, USA). In the next step, the nitrocellulose membranes slowly incubated with a rabbit polyclonal secondary antibody to mouse IgG, HRP (dilution 1:5000, 60 min) after several washes with TBST solution. The labeled proteins were distinguished by a chemiluminescence WB system. The expression of detecting proteins was semi-quantified by Image J analysis software and normalized to β-actin.

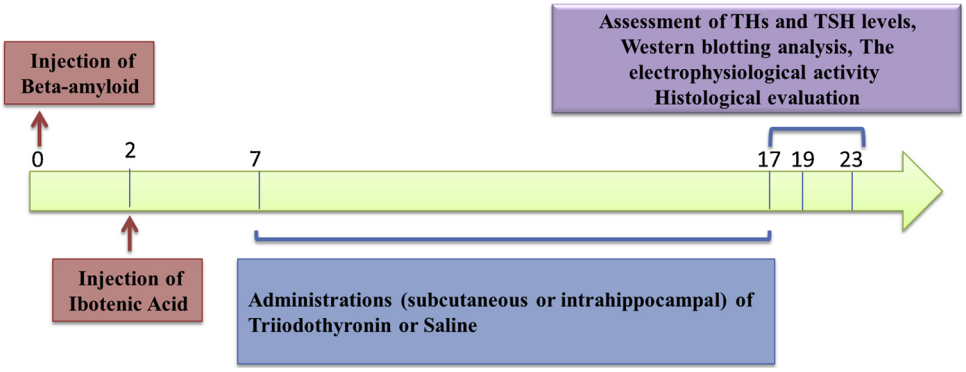

Fig. 1. The time-line scheme of experiment design.

2.9. Time line of the study

The timeline scheme was shown in Fig. 1, in order to facilitate the procedure understanding.

2.10. Statistical analysis

The collected data are presented as the Mean ± SEM, were analyzed with one-way ANOVA and followed by Tukey's post hoc test. Differences with P values of less than 0.05 were considered statistically significant.

3. Results

3.1. Effects of interahippocampal injection of Aβ and treatment with T3 on body mass of AD rats

As revealed in Fig.2, the body mass in AD rats was declined significantly compared with the Sh-O + Veh group (**P < 0.01), and significantly increased after S.C., and I. H injections of T3 in AD-treated rats compared with the AD group ($^{\#\#}$P < 0.001, $^{\#\#}$P < 0.001).

3.2. S. C and I. H injections of T3, improved PS Amp in TBI rats

As shown in Fig. 3.A–B, the average percent of PS Amp (mv) and slope of fEPSP (v/s) decreased significantly (p < 0.001, p < 0.001) in AD rats during all LTP recording times after HFS when compared with

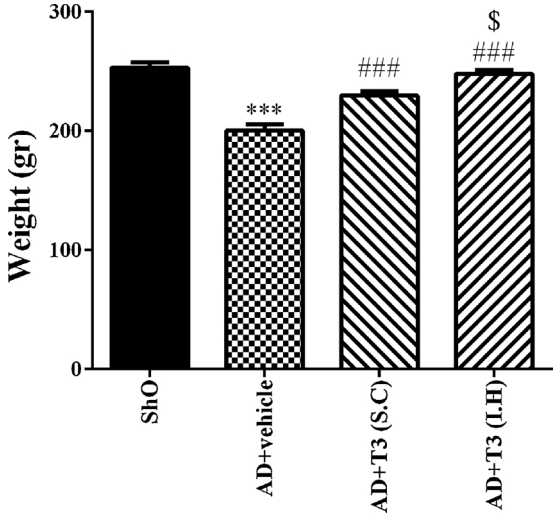

Fig. 2. Effect of central and peripheral injections of T_3 on body weight of AD rats (**P < 0.01 vs. Sh-O, $^{\#\#}$P < 0.01 vs. AD + vehicle, $^{\$}$P < 0.01 vs. AD + T3 (S.C), n = 8, one way ANOVA followed by Tukey's Post hoc test).

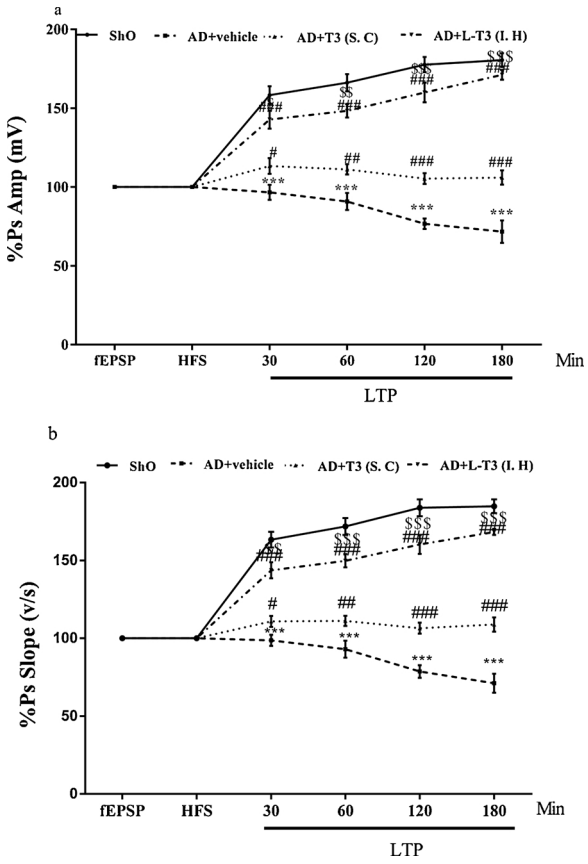

Fig. 3. (A): Percentages of PS Amp, (B): Percentages of fEPSP slope. Two-way ANOVA repeated measurements, followed by Tukey's Post hoc test (n = 8). (*** P < 0.001 vs. Cont, $^{\#\#}$ P < 0.01, and $^{\#\#\#}$ P < 0.001 AD + T3 (S. C) and (I. H) + vs. AD + vehicle).

ShO group, while it was amplified significantly (p < 0.001) in same times in AD rats treated with T3 (S. C and I. H) when compared AD rats (p < 0.01, p < 0.001).

3.3. S. C and I. H injections of T3 regulated the level of T_4, T3 and TSH in the hippocampus of AD rats

As shown in Table 1, the hippocampal concentration of T_4, T3, FT3 and FT4 were significantly declined in AD animals compared with the Sh-O groups (**P < 0.01 and ***P < 0.001, respectively). The hippocampal level of these hormones significantly increased in S. C and I. H injections of 25 μg and 50 pmol/μl T3 in AD + T3 groups compared with AD groups ($^{\#\#}$P < 0.01 and $^{\#\#\#}$P < 0.001, respectively). In all AD animals, compared with the Sh-O animal group, hippocampal level

Table 1
Hippocampal levels of thyroid hormones and thyroid-stimulated hormone (TSH) in different groups.

Factors					
Groups T4(μg/dL)	FT_4I T3 (μg/dL)	FT_3I TSH (μIU/mL)			
ShO	5.12 ± 0.02	2.23 ± 0.35	1.96 ± 0.15	0.85 ± 0.02	1.16 ± 0.2
AD + Vehicle	1.49 ± 0.028 **	0.60 ± 0.12**	1.47 ± 0.35**	0.65 ± 0.01*	0.55 ± 0.25*
AD + T3 (S. C)	2.82 ± 0.03$^{\#}$	1.24 ± 0.12$^{\#}$	1.00 ± 0.22	0.44 ± 0.14$^{\#}$	$^{\#}$ 0.83 ± 0.1$^{\#}$
AD + T3 (I. H)	5.94 ± 0. 2$^{\#\$\$\$}$	2.60 ± 1.23$^{\#\#\$\$}$	1.91 ± 0.18$^{\#\#\$}$	0.85 ± 0.014$^{\#\#\$}$	…1.19 ± 0.02$^{\#\$}$

Note: AD: Alzheimer's disease; L-T4: levothyroxine; T4: thyroxine; FT4I: free thyroxine index; T3: triiodothyronine; FT3I: free triiodothyronine index (*P < 0.05 and **P < 0.01 vs. Sh-O, $^{\#}$P < 0.05 and $^{\#\#}$P < 0.01vs. AD + vehicle, $^{\$}$P < 0.01 and $^{\$\$}$P < 0.01 vs. AD + T3 (S.C), n = 8, one-way ANOVA followed by Tukey's post hoc test).

of TSH was significantly declined (**P < 0.01, Table 1) and significantly improved in AD animals treated with T3 compared to AD animals ($^{\#}$P < 0.01,$^{\#\#}$P < 0.01 vs. AD + vehicle, $^{\$}$P < 0.05 and $^{\$\$}$P < 0.01 vs AD + T3 (S. C), n = 8, one way ANOVA followed by Tukey's Post hoc test).

3.4. S. C and I. H injection of T3, improved protein expressions of BDNF and reelin in the hippocampus of AD rats

In AD animal groups, the expression of BDNF and reelin proteins was significantly weakened compared with the Sh-O animal groups (***P < 0.001 and ***P < 0.001, respectively). The expression of these proteins was significantly enlarged in S.C. and I.H administrations of 25 μg and 50 pmol/μl T3 in AD-treated rats ($^{\#\#}$P < 0.01 and $^{\#\#\#}$P < 0.001, respectively) (Figs. 4 A–B).

3.5. Histological evaluation

In this research, was used the hematoxylin and eosin staining method, to evaluate of Aβ plaques in the periventricular (PV) region of brain tissue. These plaques were gradually diffused in the total brain after AD induction by Aβ plus ibo as shown in (Fig. 5B). Both I. H and S. C administration of T3, improved the histological injury and reduced the accumulation of Aβ plaques in the PV area of the brain (C and D in Fig. 5) compared to A section from the AD rats).

4. Discussion

The results of the present study showed that bilateral infusion of Aβ1-42 plus Ibo into the DG caused by loss of body mass, decrease of brain levels of THs and TSH as well as a decline in BDNF and reelin protein expressions in AD rats. The S.C., and I.H injections of triiodothyronine associated with higher levels of T4, T3 and TSH and increased their body mass. Also, each two type injection of T3 increased BDNF and reelin expression in the hippocampus of AD rats. Besides, I. H microinjection of T3 had more significant effects compared with S. C administration in AD rats.

Our results proposed that bilateral Aβ plus Ibo injection in the hippocampus caused reduction in body weight and the result is in agreement with previous research (Intebi et al., 2002).

The progressive accumulation of Aβ plaque results to increase of neurotoxicity and oxidative damage. Previously evaluation indicated that Aβ induced-neurotoxicity is regulated by the intracellular accumulation of ROS, oxidative stress, mitochondrial dysfunction and apoptosis (Bastianetto, Yao, Papadopoulos, & Quirion, 2006; Liu et al., 2011). Accordingly, Aβ peptide accumulation is known as one of the main reasons of AD pathology (Shabani et al., 2017). Also, in a recent study showed that ibotenic acid (as a glutamate receptor agonist result to excitotoxicity in hippocampal cholinergic neurons (Ogino et al., 2014). Moreover, evidence demonstrated that damage of cholinergic neurons in the DG of hippocampus may contribute to memory and learning impairment associated with neurodegenerative disease like AD (Pepeu, Grossi, & Casamenti, 2015). In this study, Ibo in order to induce more neurotoxicity and advanced step of AD, was used. In our previous reports, it was shown that Intrahippocampal administration of Aβ plus Ibo resulted in a spontaneous discharges impairment of neurons and the memory process disorder (Shabani et al., 2016). Histological studies in AD exhibited neuronal circuit damage involved in memory performance, namely the hippocampus, which appears to be preceded by synaptic and neuronal dysfunction (Ferreiro et al., 2012).

Experimental investigations indicated that the CNS has severe requirements for THs (same known hormones). Besides, the content of THs in whole brain has a tendency to be kept within a narrow range even in the presence of small fluctuations of circulating thyroxin level (Dratman, Crutchfield, Gordon, & Jennings, 1983). In previous reports, has shown a direct link between thyroid gland dysfunction with cognitive impairment and the pathogenesis of AD, comprising beta amyloid plaques deposition and neuronal death. Both hypothyroidism and hyperthyroidism may be associated with some of the clinical features of AD (Davis et al., 2008; Kalmijn et al., 2000). Thus, even small alterations in the brain content of THs may have important cognitional and behavioral effects (Loosen, 1992). The several studies showed THs prevented cognitive impairment and improved the neurological function in AD animal model by decreasing the choline-acetyl transferase (ChAT) activity and increasing the level of each, protecting against the damage of free radicals, and increasing the number of neurons in the hippocampus of AD mice (Fu et al., 2010; Shabani et al., 2016). Thyroid response elements (TRE) were also found in the APP gene and T3 negatively modulated APP gene expression by repressing the APP promoter in cultured neurons of rat (Contreras-Jurado et al., 2012). Administration T3 also changes the splicing of APP gene and secretion of its various isoforms, leading to different alterations in the expression of Aβ (Latasa, Belandia, & Pascual, 1998). In addition, transthyretin (a transport protein for T4 in cerebrospinal fluid (CSF)) decreased in the CSF of AD patients and therefore thyroxine transport into the brain decreased in AD (Merched et al., 1998). Apolipoprotein E (apoE), a protein generally involved in lipid metabolism, is associated with numerous neurodegenerative disorders such as AD (Roman et al., 2015). Besides, pervious results indicated a close relationship between transthyretin, apolipoprotein (ApoE), and actin, which proposed a potential metabolic role of ApoE genetics and transthyretin in cytoskeleton structure of neurons that may be linked with AD pathogenesis (Merched et al., 1998). Roman et al. showed a key role of THs and their nuclear receptors in apoE gene expression regulation in astrocytes. They revealed that THs improved apoE gene expression in astrocytes dose-dependently (Roman et al., 2015). In addition, T4 conversion to T3 is adjusted by type II deiodinase. Laboratory Reports showed that the type II deiodinase and ultimately, T3 levels declined in the brain of AD rats (Kapaki et al., 2006). Hence, it seems that both disturbances in T4 transport and disturbance in deiodinase activities result in a decrease in the brain levels of T3 and T4. Davis and colleagues showed lower T3 levels in prefrontal cortex of post-mortem brains of AD patients (Davis et al., 2008). Our experimental results showed that intrahippocampal administration of Aβ and Ibo caused a significant decrease in T4 and T3

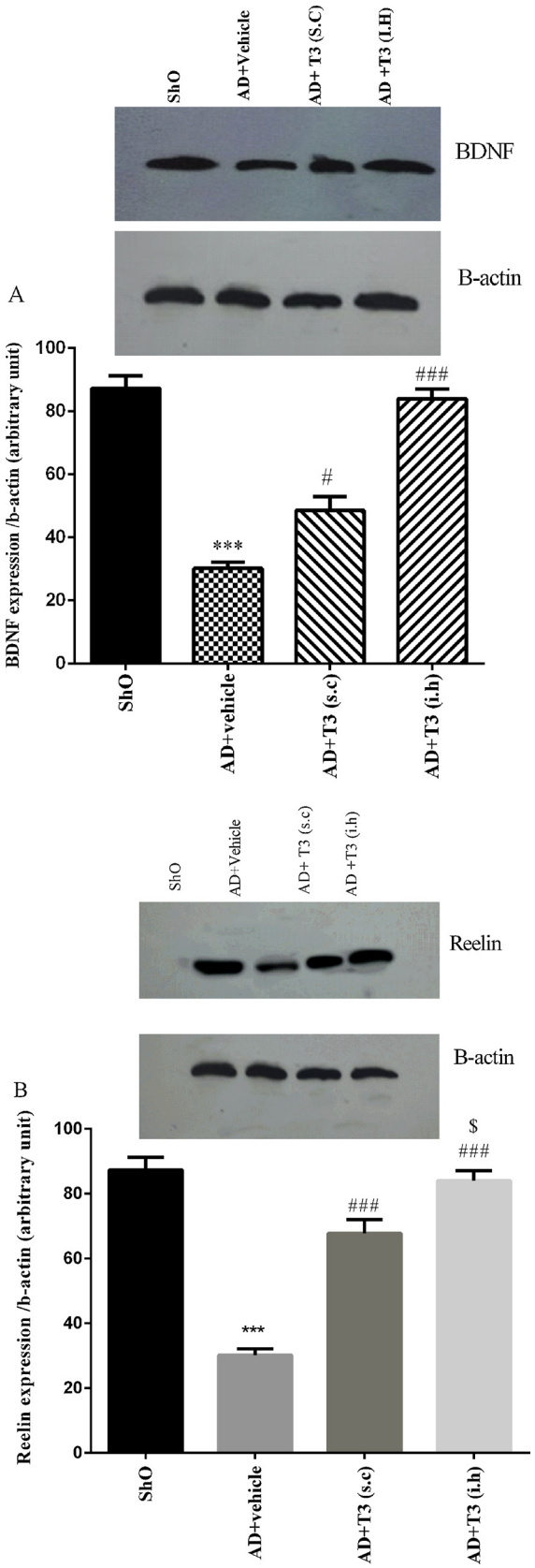

Fig. 4. Effect of triiodothyronine on BDNF and reelin expression in different groups (A–B). The results showed that central and peripheral injections of T3 increased BDNF and reelin protein expression in AD rats (***P < 0.001 vs. Sh-O, ##P < 0.01 and ###P < 0.001 vs. AD + vehicle, $P < 0.05 and $$P < 0.01 vs AD + T3 (S.C), n = 8, one way ANOVA followed by Tukey's Post hoc test).

levels in the hippocampus region of the brain.

Despite the possible relationship between HPT abnormalities and dementia, the precise nature of the link is unclear. Scientist's findings in AD patient population revealed HPT abnormalities comprising alteration in serum TSH levels (both lower and higher) associated with risk increased in AD (Luboshitzky, Oberman, Kaufman, Reichman, & Flatau, 1996). Our findings revealed lower TSH level in AD group compared to other groups and these results is in agreement with previous studies (Kalmijn et al., 2000; Luo, Yano, Mao, Jackson, & Stopa, 2002), whether altered TSH levels occur in AD neuropathology is unclear. Thyrotropin releasing hormone (TRH) is a neuropeptide that regulates TSH release of anterior pituitary. Lately evidences have shown that TRH is depleted in the hippocampus of post-mortem AD. The same researchers showed that the secretion of TRH by the hypothalamus or pituitary responsiveness to TRH decreased in the hippocampus of AD and hence, the level of TSH and thyroxine declined in the hippocampus. TRH depletion in hippocampus itself may lead to AD pathology by increasing phosphorylation of tau proteins (Luo et al., 2002). Thus, low levels of TSH may be the consequence of TRH depletion, rather than the cause of, AD. However, more precise studies required for evaluation of the link between HPT- axis abnormality and AD pathology.

The hippocampus has a higher neuroanatomical expression of BDNF and reelin in the brain. For this reason, the hippocampus is a known region for its high degree synaptic plasticity (Lee & Son, 2009; Numakawa et al., 2010). Various findings revealed that BDNF had an important role in regulation phosphorylation trafficking and expression of N-methyl-D-aspartate receptor (NMDAR) subunits. Also, it is documented that BDNF facilitate Ca^{2+} influx and stimulate intracellular signaling pathway involved in long term potentiation by direct implications on NMDAR subunits (Mulholland, Luong, Woodward, & Chandler, 2008; Numakawa et al., 2010; Wang et al., 2014). Moreover, another study proposed that Aβ-induced neuronal death may be a consequence of BDNF deficiency (Meng, He, & Xing, 2013). Another study indicated that brain levels of BDNF protein expression were lower in AD patients (Buchman et al., 2016). Additionally, higher hippocampal BDNF expression associated with slower cognitive impairment and might also decrease the damaging effects of AD pathology on cognitive defects. It has been shown that cognitive impairment exhibited by AD mouse can be rescued through a BDNF delivery (Nagahara et al., 2009).

Reelin, an extracellular matrix protein adjusts synaptic plasticity in the overall brain, thereby favoring memory and learning, formation (Botella-López et al., 2009). Most studies have shown an over expression of reelin in populations of neurons improved the number of synapses in the hippocampus (Pujadas et al., 2014). However, mice that exhibited decreased brain levels of reelin protein expression have fewer hippocampal synapses (Niu, Yabut, & D'Arcangelo, 2008). Besides, reelin improved LTP and protected against Aβ-induced neurotoxicity and deficiency (Durakoglugil, Chen, White, Kavalali, & Herz, 2009). Several findings showed a reduction in the protein and mRNA level of reelin in the entorhinal cortex of AD mouse and aged rats that are cognitively impaired (Chin et al., 2007; Stranahan, Haberman, & Gallagher, 2011). Consistent with these results, we exhibited that reelin and BDNF protein expression diminished after induction of AD by administration of Aβ1-42 plus Ibo. Another finding indicated that reduction in expression of reelin in AD mice accelerated amyloid-Plaque formation (Chin et al., 2007), while the administration of reelin delayed amyloid-beta fibril formation and rescued memory and learning deficits in a model of AD (Pujadas et al., 2014).

BDNF plays very important role in regulating synaptic transmission and plasticity (Mattson et al., 2004; Murer et al., 2001). Several lines of evidence declared that the reduction of T_4, T_3 and FT_4 serum levels down-regulated mRNA and protein expression of BDNF in the hippocampus in male rats. In addition, temporary postnatal treatment of rats with T4 and T3 increased BDNF protein and mRNA levels in the hippocampus (Lüesse et al., 1998). It was revealed that injection of T3

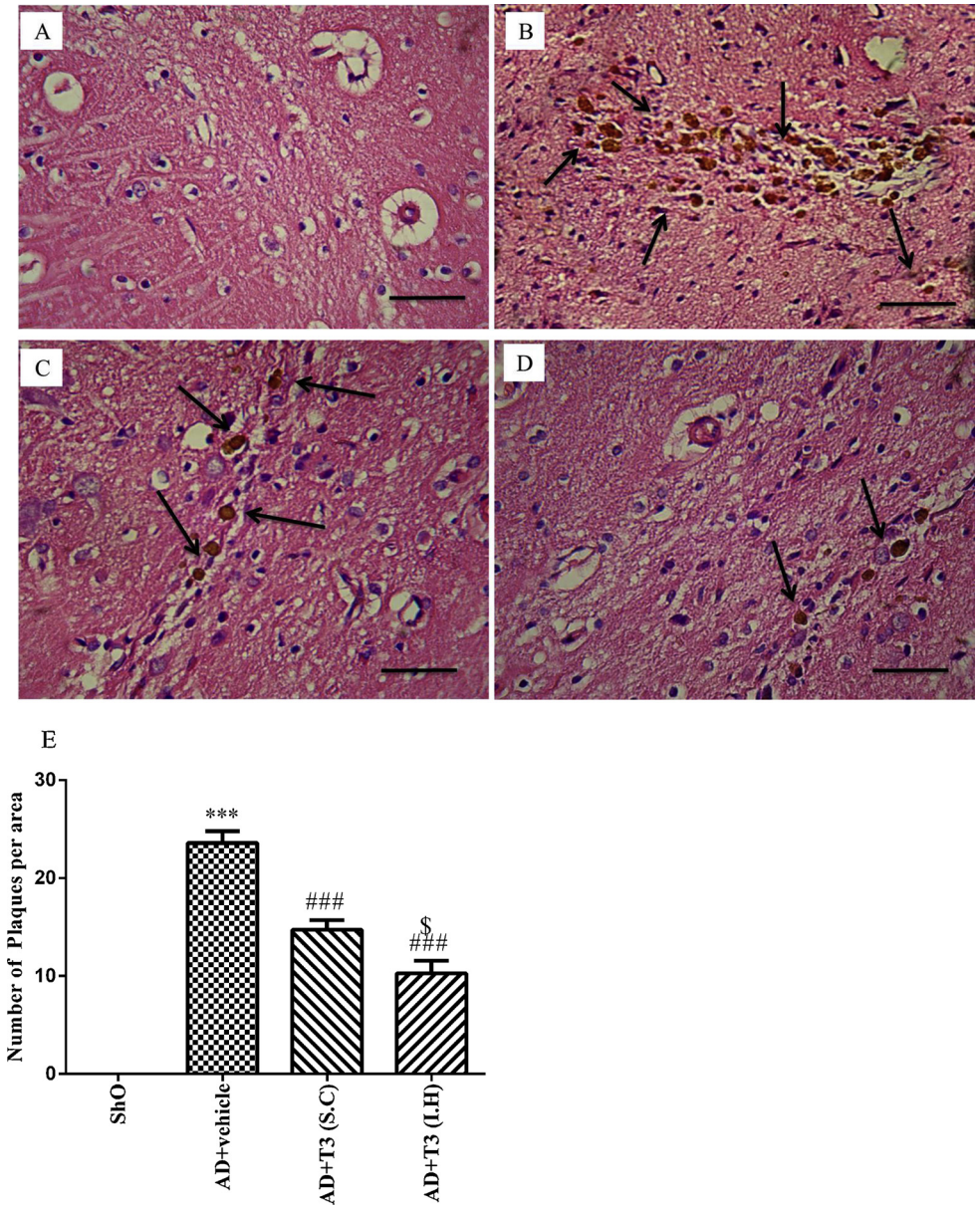

Fig. 5. (A–D) Effect of injection of Aβ plus Ibo and treatment with T3 (both S. C and I. H) on histopathology of the PV region of the brain (presented by the arrows, hematoxylin and eosin, scale bars = 100 μm). (A) ShO, (B) AD + vehicle, (C) AD + T3 (S.C) and (D) AD + T3 (I.H). (E) Number of plaques in the PV area. ***P < 0.001 vs. ShO, ###P < 0.01 and ###P < 0.001 vs. AD + vehicle, $$$P < 0.01 vs AD + T3 (I.H), n = 5, one way ANOVA followed by Tukey's Post hoc test).

increased hippocampal reelin and BDNF protein expression (Sui & Li, 2010). The significant down-regulation of reelin observed under thyroid hormones deficiency was restored on thyroxin treatment, proposing a direct relationship between thyroid gland status and molecular cues that govern neuronal maturation and migration (Pathak, Sinha, Mohan, Mitra, & Godbole, 2011). The present research indicated; for the first time, that T3 administration (both S. C and I. H), increased expression of BDNF and reelin in the hippocampus of AD rats.

5. Conclusions

In conclusion, the results of this study revealed that inter-ahippocampal administration of Aβ1–42 plus Ibo caused pituitary-thyroid abnormalities in AD rats, including decrease of THs and TSH levels. These thyroid gland abnormalities associated with a reduction of BDNF and reelin protein expression in AD rats. Treatment of AD model rats with T3 (both S. C and I. H), increased expression of BDNF and reelin by the regulation of hippocampal THs and TSH levels. Also, I.H. microinjection of T3 was more effective than S.C.

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Ethical statement

All animal experiments were totally done in accordance with the principles set by the Local ethics committee (Ethics Code: IR.AJUMS.REC145).

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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