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Alterations in Expression of Neurodegeneration-Related Genes After Long-Term Potentiation in the Hippocampus of Hyperthyroid Rats

Hipertiroidili Sıçanların Hipokampüsünde Uzun Dönemli Güçlenme Sonrası Nörodejenerasyonla İlgili Genlerin Anlatımındaki Değişiklikler

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ABSTRACT

Aim: Imbalances in plasma thyroid hormone levels cause changes in synaptic response, resulting in impairments in learning and memory. It is not well understood how the excess of these hormones (T3 and T4 hormones), which modulate gene transcription, affects gene expression after high-frequency stimulation (HFS). Therefore, in this study, it was aimed to investigate the transcriptional changes accompanying long-term potentiation (LTP) in genes related to neurodegeneration in rats with hyperthyroidism.

Materials and Methods: 12 male Wistar-albino rats were used in the study. The rats were divided into two groups as control and hyperthyroidism groups. To induce hyperthyroidism, L-tyroxine was administered intraperitoneally at a dose of 400 µg/kg/day for 21 days, starting on the 39th day. To investigate LTP, when rats were 60-day-old, LTP was induced by giving HFS at 100 Hz for 1 second at 5-minute intervals after 15 minutes of basal recording to the perforating pathway and the excitatory postsynaptic potential (EPSP) slope and population spike (PS) amplitude were recorded in the granule cell layer of the dentate gyrus. The mRNA levels of genes related to neurodegeneration in stimulated hippocampus were measured by reverse transcription-polymerase chain reaction method.

Results: After HFS, LTP was induced in the control group at the EPSP slope, while LTP was not induced in the hyperthyroid group. However, LTP was induced at the PS amplitude in both groups. In hyperthyroid rats, Gsk3β, P35(Anxa) Calpn1 and Bace1, Psen2-mRNA levels were not significantly different compared to the control group, while Akt1, Cdk5 and Mapt-mRNA levels were found to be increased significantly compared to the control group ($p<0.05$).

Conclusion: These findings suggest that the excess of thyroid hormone during neuronal plasticity partially alters the expression of genes related to neurodegeneration.

Keywords: Hippocampus, hyperthyroidism, neuroplasticity, gene expression

ÖZ

Amaç: Plazma tiroid hormon (T3 ve T4) düzeyindeki dengesizlikler sinaptik yanıtta değişikliklere neden olarak öğrenme ve bellekte bozulmalara neden olur. Gen transkripsiyonunu modüle eden T3-T4 hormon fazlalığında, yüksek frekanslı uyarı (YFU) sonrası gen anlatımının nasıl etkilendiği çok iyi anlaşılmamıştır. Çalışmamızda, hipertiroidi oluşturulan sıçanların hipokampüsünde uzun dönemli güçlenmenin (UDG) indüklenmesi ile plastisite indüksiyonuna eşlik eden, nörodejenearatif proteinlerle bağlantılı olan, amiloid prekürsör protein (APP) ilişkili (Bace1 ve Psen2) ve tau fosforilasyonuyla ilişkili proteinlerin (Gsk3β, Cdk5, Akt1, Mapt P35(Anxa) ve Calp1) mRNA düzeyindeki değişiminin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Sıçanlar kontrol ve hipertiroidi grupları olarak ikiye ayrıldı. Hipertiroidi grubu sıçanlara yaşamlarının 39. gününden başlanarak 21 gün süresince 400 µg L-tiroksin/kg/gün intraperiteonal (ip) yolla verildi, kontrol grubuna ise aynı şartlarda serum fizyolojik verildi. Sıçanlar 60 günlük olduklarında, test uyaran şiddet belirlenmesi, bazal kayıt alınmasının ardından 5 dakika arayla 1 saniye boyunca 100 Hz YFU verilerek UDG

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indüklendi ve dentat girus granül hücre tabakasında eksitatör postsinaptik potansiyel (EPSP) eğimi ve popülasyon spike (PS) genliği kaydedildi. Bazal kaydın EPSP, PS ortalama değerleri 100 kabul edildi, UDG'nin indüksiyon ve idame dönemlerine ait EPSP ve PS değerleriyle karşılaştırıldı. İstatistiksel analizler için Mann-Whitney U testi kullanıldı. Uyarılmış hipokampüslerdeki APP ilişkili ve tau fosforilasyonuyla ilişkili proteinlerin mRNA seviyeleri real-time-polimeraz zincir reaksiyonu yöntemiyle ölçüldü.

Bulgular: Kontrol grubunda YFU verilmesini takiben EPSP eğiminde UDG indüklenirken hipertiroidi grubunda UDG indüklenmedi. Her iki grupta PS genliğinde UDG indüklendi. Hipertiroidili sıçanlarda, Gsk3β, P35(Anxa) Calpn1 ve Bace1, Psen2-mRNA seviyesi kontrol grubuna göre anlamlı düzeyde farklılık göstermezken, Akt1, Cdk5 ve Mapt-mRNA seviyeleri kontrol grubuna göre anlamlı düzeyde arttı ($p<0,05$).

Sonuç: Bu bulgular, tiroid hormon fazlalığının, nöronal plastisite sırasında nörodejenerasyonla ilgili genlerin ekspresyonunu kısmen değiştirdiğini göstermektedir.

Anahtar Kelimeler: Hipokampüs, hipertiroidizm, nöroplastisite, gen ifadesi

INTRODUCTION

Thyroid hormones have genomic and non-genomic effects. Thyroid hormones cause the activation of intracellular PI3K-Akt/Gsk3β and Mapk signaling pathways by interacting with the specialized membrane proteins monocarboxylate transporter 8 and integrin αVβ3, which are involved in the communication between the extracellular and intracellular environment, with their non-genomic effect. The non-genomic effects of thyroid hormones are short term[1-4]. The main effect of thyroid hormone is genomic, it binds to its receptors in the nucleus and initiates various transcription events and directly affects the biological functions of the cell[1]. Changes in hormone level can affect the expression levels of genes associated with synaptic plasticity and neurodegenerative diseases. The underlying cause of the majority of neurodegenerative diseases is based on defects in gene expression. In recent years, studies performed to explain the causes of neurodegenerative diseases have focused on changes in gene expression[5,6]. The hippocampus is considered one of the most important brain regions affected by these diseases. In the previous studies conducted in our laboratory, it was determined that high or low thyroid hormone levels caused significant changes on hippocampal synaptic plasticity forms[7,8].

Long-term potentiation (LTP) means permanently increasing the strength and number of synaptic connections, depending on the increase in the frequency of neural activity from a presynaptic neuron or nerve pathway, and is a form of synaptic plasticity that is the most studied and well-known. A growing number of studies on synaptic plasticity have shown that LTP not only strengthens synaptic connections during learning, but also promotes the formation of new neurons in the hippocampus[9,10]. LTP requires the phosphorylation of some critical kinases[11]. At the molecular level, the effect of an increase in kinase or phosphatase activity is relatively short term, and longer maintenance of these changes requires gene transcription and synthesis of neuronal proteins[12].

Changes in the expression of certain genes following the induction of the forms of synaptic plasticity that underlie learning and memory allow these forms of plasticity to persist through protein synthesis[13]. On the other hand, the over- or under-synthesized protein products formed by genes with changed expression in thyroid hormone deficiency or excess may cause tau and amyloid beta proteins, which are associated with some neurodegenerative diseases, to be synthesized and phosphorylated at different levels. As a result, the incidence of neurodegenerative diseases such as Alzheimer's disease, characterized by dementia, may increase.

In our study, it is aimed to investigate the change in mRNA level of amyloid precursor protein (APP)-associated (Bace1 and Psen2) and tau phosphorylation-related proteins (Gsk3β, Cdk5, Akt1ve Mapt, P35(Anxa), Capn1), associated with neurodegenerative proteins and accompanying the induction of LTP in the hippocampus of hyperthyroid induced rats.

METARIALS AND METHODS

Experimental Animals

This study was carried out within the scope of the project numbered TDK-2019-9405 supported by Erciyes University Scientific Research Projects Unit and with the approval of Erciyes University Animal Experiments Ethics Committee dated 17.07.2019 and numbered 19/133. For the study, 12 Wistar albino male rats, weighing 180-200 gr, produced in Erciyes University Hakan Çetinsaya Experimental and Clinical Research Center, were used. Ethical principles were taken into account in the study in order not to use unnecessary experimental animals and not to cause pain on experimental animals. Rats were divided into two groups, as the control group (n=6) and the hyperthyroidism group (n=6).

Creating Hyperthyroidism

To induce hyperthyroidism (n=6), 39-day-old animals were given 400 µg/kg/day L-thyroxine (Sigma, catalog number: T2501-5G, 0.5 mL) intraperitoneally (ip) for 21 days[14]. The control group (n=6) rats were given saline ip in the volumes given to the experimental group rats for 21 days starting from the 39th day.

Electrophysiological Recording

After rats were anesthetized with urethane (1.2 g/kg), they were placed in stereotaxis using ear and mouth sticks. The stimulating electrode was lowered into the hippocampus according to the coordinates of anteroposterior (AP): 6.5 and mediolateral (ML): 3.8 mm, and the recording electrode according to the coordinates of AP: 3 mm ML: 2.13 mm, and the two-channel glass micropipette filled with 3 M NaCl was lowered into the hippocampus until a typical response was observed.

The perforating pathway was stimulated with stimuli varying in the range of 0.1-1.5 mA with the stimulus electrode, and the stimulus intensity (test stimulus intensity), which constituted half of the maximum intensity of the responses received from the dentate gyrus granule cells, was determined by input/output experiments. The basal record of the experiment was recorded for 15 minutes. During the basal recording, the perforating pathway was stimulated with this determined stimulus intensity every 30 seconds, and the excitatory postsynaptic potential (EPSP) slope, field potentials in the dentate gyrus granule cell layer, and population spike (PS) amplitude, were recorded for 15 minutes. For the LTP response, a stimulus was given every 30 seconds, followed by a 15-minute basal recording, and by induction with high-frequency stimulation (HFS) at 100 Hz for 1 second at 5 minutes intervals (15 minutes in total). EPSP slope and PS amplitude were recorded by continuing the stimulation with a test stimulus every 30 seconds for a total of 60 minutes following HFS stimulation.

After the electrophysiological recordings were taken, the blood of the rats were taken intracardiacly, then they were decapitated, and their brains removed, and their hippocampi were isolated. The collected blood was centrifuged and the plasma was stored at -20 °C until the study day. Isolated hippocampi were stored at -80 °C until real-time PCR (rt-PCR) analysis. The level of fT4 in plasma and mRNA levels of APP-related (Bace1 and Psen2) and tau phosphorylation-related proteins (Gsk3β, Cdk5, Akt1 P35(Anxa), Calpn1 and Mapt) in hippocampus tissue were measured.

Measuring Plasma Free T_4 Levels

After collecting plasma samples from all groups, plasma fT4 levels were measured in a plate reader (Multiskan™ FC Microplate Photometer) with a commercial ELISA kit (Cloud Cone Corporation, USA).

Real-Time-PCR Measurements

RNA Isolation from Hippocampus Tissue

Hippocampus tissues taken into tubes each containing 1000 µL of RNA isolation solution (PureZOL™: BioRad, USA) were homogenized with the help of a homogenizer. They were then incubated at room temperature for 5 min. 400 µL of chloroform was added to them and vortexed for 15 seconds. They were incubated for 15 min at room temperature and centrifuged at 14000 g for 20 min at +4 °C. The aqueous phase formed at the end of centrifugation was taken into a new eppendorf tube. 300 µL of isopropanol was added to it (depending on the amount of aqueous phase) and inverted several times. It was incubated at room temperature for 10 minutes and centrifuged at 14000 g for 30 minutes at +4 °C. The supernatant obtained at the end of centrifugation was discarded. 1 mL of 75% ethanol was added to the pellet portion at the bottom and vortexed. Afterwards, it was centrifuged at 7500 g for 5 min at +4 °C. The supernatant formed at the end of centrifugation was discarded and the ethanol around the pellet was pipetted and cleaned. The pellet was dried at room temperature for 10-15 minutes until the ethanol evaporated. After drying, 20 µL of NFW (Nuclease Free Water) was added and the pellet was resuspended. It was then placed in ice and kept in a refrigerator at +4 °C for 10-15 minutes. At the end of this period, RNA concentration (ng/µL) was measured in the nanodrop.

cDNA Synthesis

Before starting the study, all RNA samples from rats were numbered and these numbers were written on the PCR tubes. The iScrip™cDNA synthesis Kit (1708890, BioRad, USA) was used for cDNA synthesis. cDNA synthesis was performed according to the iScrip™cDNA Kit protocol. In order to create a reaction with equal amount of RNA in each sample, the amounts of RNA and water to be added were calculated according to the RNA quantification results. Desired amount of NFW and RNA samples were added to the PCR tubes with a total volume of 15 µL. Afterwards, 5 µL of 5X reaction buffer (4 µL) and Reverse Transcriptase (1 µL) mixture was dispensed into each PCR tube and the tubes were placed in the PCR device (CFX Connect rt-PCR Detection System BioRad). At the end of PCR, 20 µL of cDNA product was formed. In the PCR device (CFX Connect rt-PCR detection System, BioRad), it was kept for the steps of the incubation, denaturation and extension and annealing for 5 minutes at 25 °C, 30 minutes at 42 °C and 5 minutes at 85 °C, respectively.

mRNA Expression Study

The mRNA expressions of Gsk3β, Akt1, Cdk5, Mapt, P35(Anxa), Calpn1, Bace1, and Psen2 genes were studied on the BIORAD CFX Connect rt-PCR instrument (BIORAD, USA), using the SsO Advanced Universal IT SYBR Green Supermix kit (10000076382, BIORAD, USA). A reaction mixture consisting of SYBR Green Supermix (10 µL), Nuclease Free Water (8 µL), Primer F (0.5 µL) and Primer R (0.5 µL) included in the kit was prepared and dispensed into the wells in on a 96-well plate, and cDNA (1 µL)

was added to each well. Then, it was placed in the CFX Connect rt-PCR Detection System PCR device.

PCR was held at 95 °C for 3 min, at 95 °C for 5 sec, and at 60 °C for 15 sec (45 cycles) and at 40 °C for 30 sec for pre-incubation, amplification, and cooling conditions, respectively. All samples were duplicated in order to avoid errors that might arise due to manipulation. *β-Actin* gene was used as house-keeping gene. Ct (thresholdcycle) values obtained at the end of the process were calculated using the $2^{-\Delta Ct}$ method and normalized.

Statistical Analysis

The slope of the EPSP wave was calculated as 20-80% of the voltage difference between the beginning of the wave and the beginning of the PS wave. PS amplitude was calculated as the mean of the first negative spike and the next positive spike. The mean slope and amplitude values of EPSP and PS of 30 field potentials triggered during the initial 15-minute baseline recording time were accepted as 100. The averages of EPSP slope and PS amplitude values were calculated from 10 field potentials recorded in the first 5-minute period after HFS and in the last 5-minute period at the end of the experiment.

A single sample t-test was used to compare the values obtained within a group with the baseline values. The non-parametric Mann-Whitney U test was used to determine whether there was significance between the groups. Significance level was accepted as $p<0.05$.

Non-parametric Mann-Whitney U test was employed to determine whether there was significance between groups in fT4 level analysis. Significance level was accepted as $p<0.05$.

The conformity of the data obtained with rt-PCR to normal distribution was evaluated with histogram, q-q graphs and Shapiro-Wilk test, and homogeneity of variance was evaluated with the Levene's test. Analysis of the data was performed using GraphPad Prism software. Statistical significance level was accepted as $p<0.05$. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software (SPSS, Chicago, IL).

RESULTS

Plasma Free T_4 Level

Plasma fT4 levels of rats belonging to control and hyperthyroidism groups were measured after electrophysiological recording (Figure 1). In the L-thyroxine group, higher plasma fT4 level was measured than in the control group ($p<0.004$). This result is an indication of the development of hyperthyroidism in rats.

Analysis of Input/Output Curves

The effect of L-thyroxine administration on the basal activity of synapses and neurons before plasticity induction was evaluated with input-output curves. The mean and standard error values of EPSP slope and PS amplitude measured against stimulus intensity were given graphically (Figure 2D; Figure 3D).

Baseline efficacy, ANOVA test results, with repeated measurements of input/output curves obtained by applying pulses of varying intensity between 0.1 mA-0.5 mA before HFS, revealed a significant increase in EPSP slopes ($F_{7.70}=6.017$; $p<0.001$ Figure 2D) and PS amplitudes ($F_{7.70}=13.460$; $p<0.001$ Figure 3D) as the stimulus intensity increased, as expected. Insignificant interaction between EPSP slope ($F_{7.70}=1.32$; $p=0.27$) and PS amplitude ($F_{7.70}=0.46$; $p=0.515$ after lower-bound correction) and stimulus intensity showed that this increase was similar in all groups. The group effect was not found to be significant for EPSP slope ($F_{1.10}=0.33$; $p=0.577$) and PS amplitude ($F_{1.10}=2.65$; $p=0.134$). These findings indicate that the efficacy of the perforating pathway-dentate gyrus synapses before the triggering of LTP with HFS was similar in the control and hyperthyroid groups.

The Effect of HFS on EPSP and PS

LTP responses were measured using EPSP slope and PS amplitude. The 5-minute period (15th min-20th min) after induction was called the post-HFS period, and the 5-minute period between 70th and 75th minutes at the end of the experiment was called the maintenance period.

With single-sample t-test, EPSP slopes of the control (154.2±16.0%, $t_5=8.261$, $p=0.000$; Figure 2B) and hyperthyroidism (148.5±18.2%, $t_5=5.993$, $p=0.002$; Figure

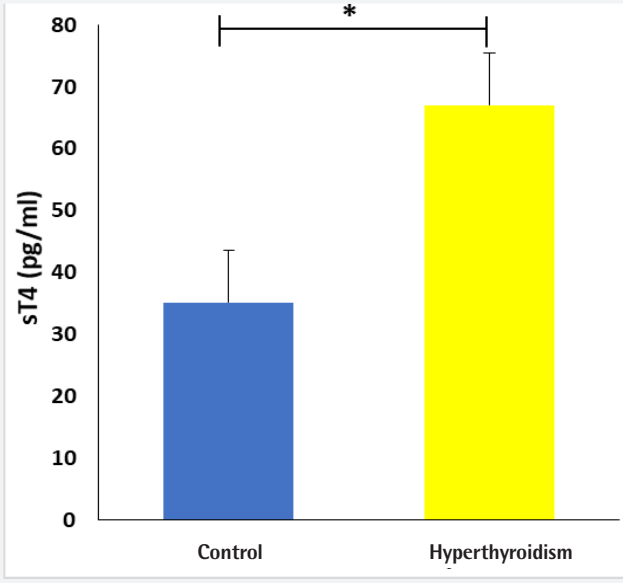

Figure 1. Plasma free T_4 values of control and experimental group rats. Values were given as mean±standard error (n=6)

**Indicates a significant difference compared to the control group (p<0.004)*

2B) groups were detected to be statistically higher after HFS compared to the recording of baseline period. During the maintenance period, EPSP slopes were found to be statistically significantly higher in the control group (119.3±9.0%, t_5=5.202 p=0.003, Figure 2C) compared to baseline recording. No statistically significant difference was found in the hyperthyroidism (109.0±12.5, t_5=1.765 p=0.138, Figure 2C) groups according to baseline recording. This finding shows us that LTP response did not occur in the synaptic component in the hyperthyroidism group.

When the differences between the groups were examined with the Mann-Whitney U test for EPSP slope, there was no statistically significant difference in EPSP slope between the groups in the post-HFS period (Z=-0.801 p=0.4) and the maintenance period (Z=-0.601 p=0.1).

When PS amplitude was evaluated considering baseline values, with single sample t-test, PS amplitudes of the control (271.0±62.4%, t_5=6.716, p=0.01; Figure 3B) and hyperthyroidism (213.47±23.2%, t_5=11.967, p=0.00; Figure 3B) groups in the post-HFS period were found to be statistically higher compared to the baseline recording. In the maintenance period, PS amplitudes of the control (212.3±21.9%, t_5=12.536, p=0.00; Figure 3C) and hyperthyroidism (182.5±35.3, t_5=5.723, p=0.02 Figure 3D) groups increased significantly compared to the baseline recording.

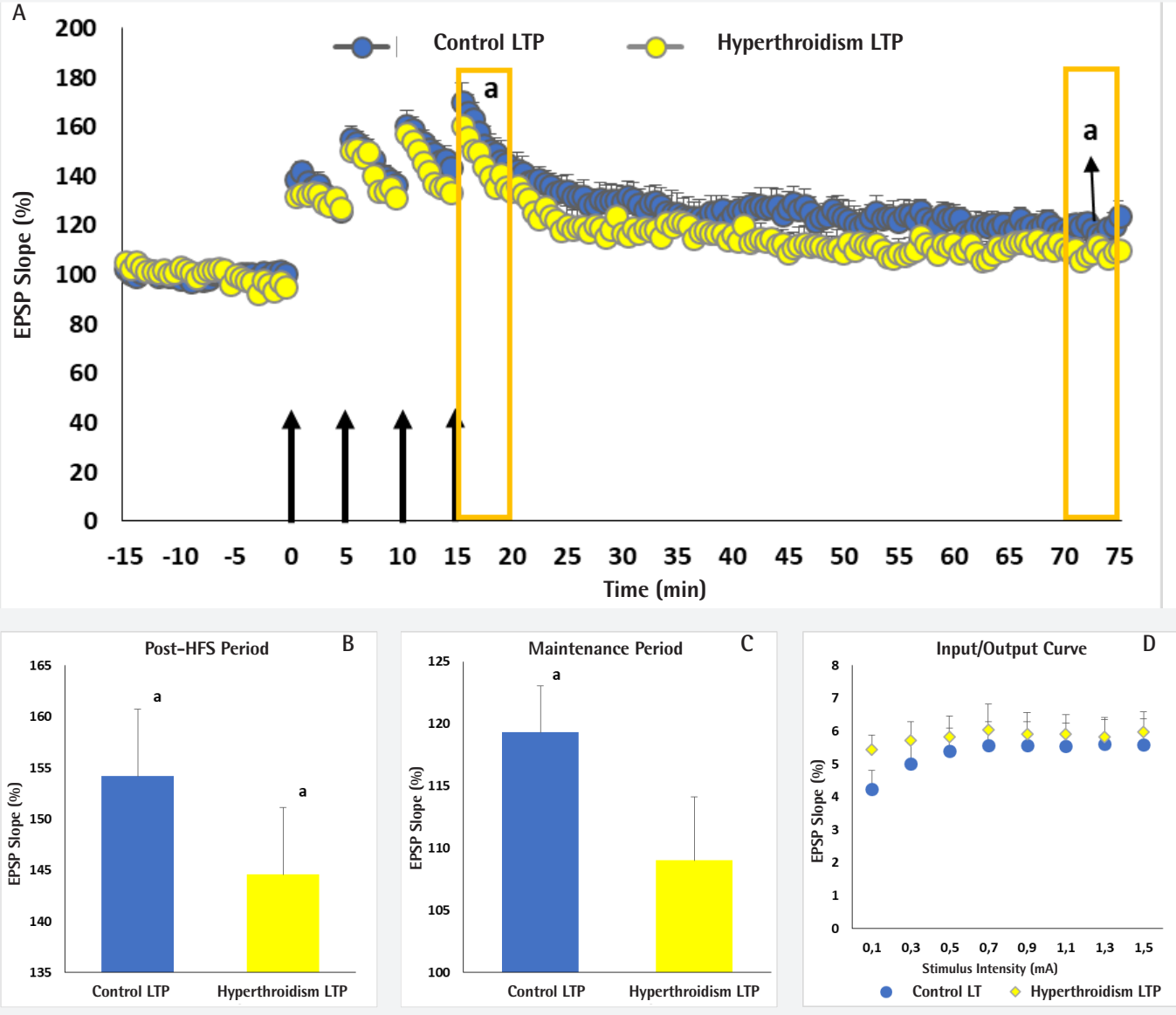

Figure 2. EPSP slopes A) EPSP slopes recorded during the experiment. B) EPSP slopes of the post-HFS period. C) EPSP slopes of the maintenance period. D) Input/output curves of EPSP slopes. [a](p<0.004) represents a significant increase compared to the baseline value

EPSP: Excitatory postsynaptic, LTP: Long-term potentiation, HFS: High-frequency stimulation

When the differences between the groups were examined for PS amplitude with the Mann-Whitney U test, no statistically significant difference was found between the groups in PS amplitude in the post-HFS period (Z=-0.922 p=0.055) and in the maintenance period (Z=-0.281 p=0.2).

Analysis of Real-Time PCR Outcomes

The mRNA expression levels of tau protein and amyloid beta precursor protein-related genes in the hippocampus, which were removed at least 60 minutes after HFS, obtained by rt-PCR method are graphically shown in Figure 4.

According to the Mann-Whitney U test, there was a statistically significant difference between the groups in the expression levels of tau-associated Akt1-mRNA (p=0.02), Cdk5-mRNA (p=0.03), and Mapt1-mRNA (p=0.006), while no statistically significant difference was found in the expression levels of tau-associated Gsk3β - P35(Anxa)-mRNA and Capn1-mRNA expression and amyloid beta precursor protein-associated Bace1-mRNA and Psen2-mRNA (p>0.05).

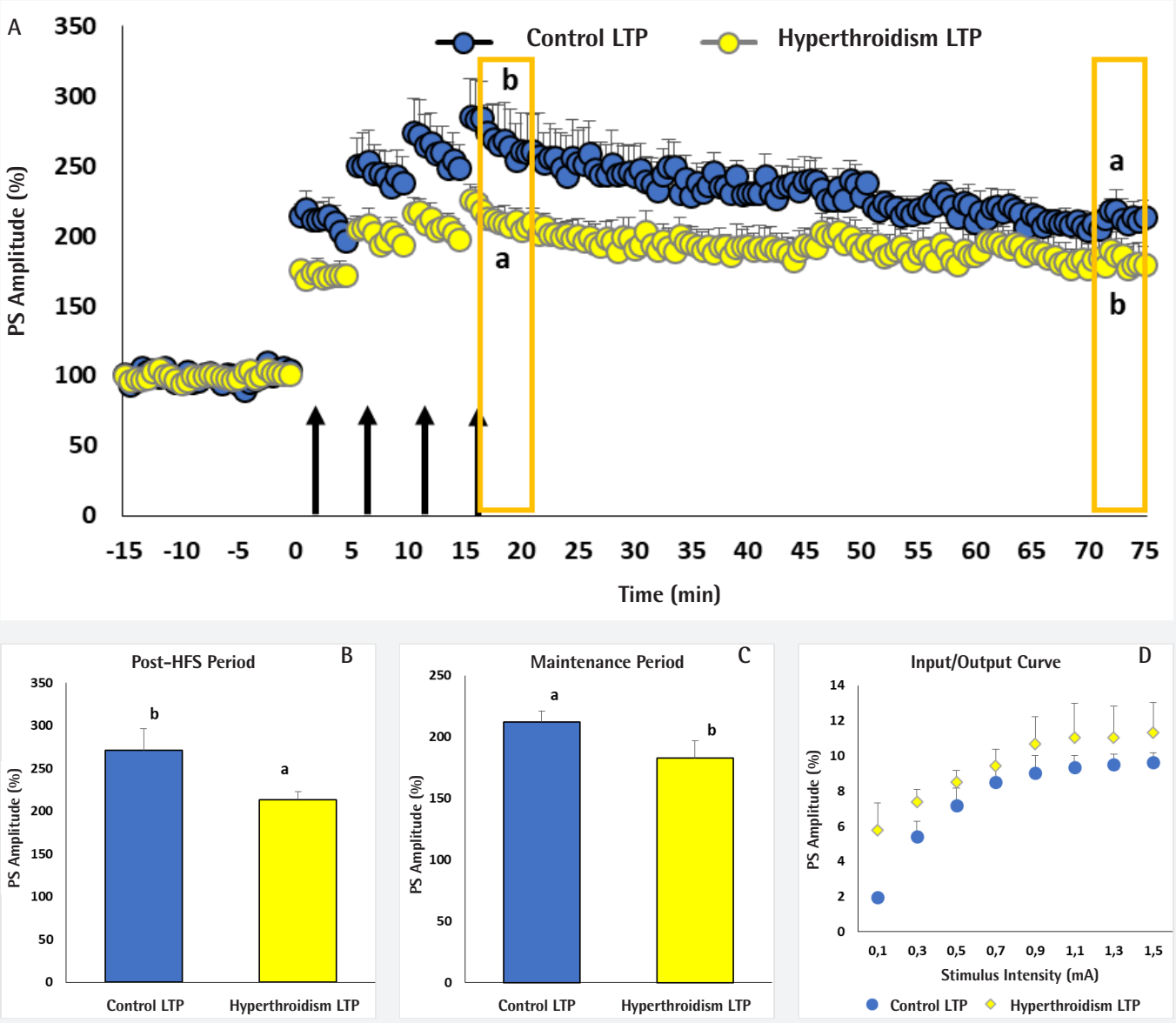

Figure 3. PS amplitudes. A) PS amplitude recorded during the experiment. B) PS amplitudes of the post-HFS period. C) PS amplitudes of the maintenance period. D) Input/output curves of the PS amplitudes. [a](p<0.004), [b](p<0.02) indicate a significant increase compared to the baseline value

PS: Population spike, LTP: Long-term potentiation, HFS: High-frequency stimulation

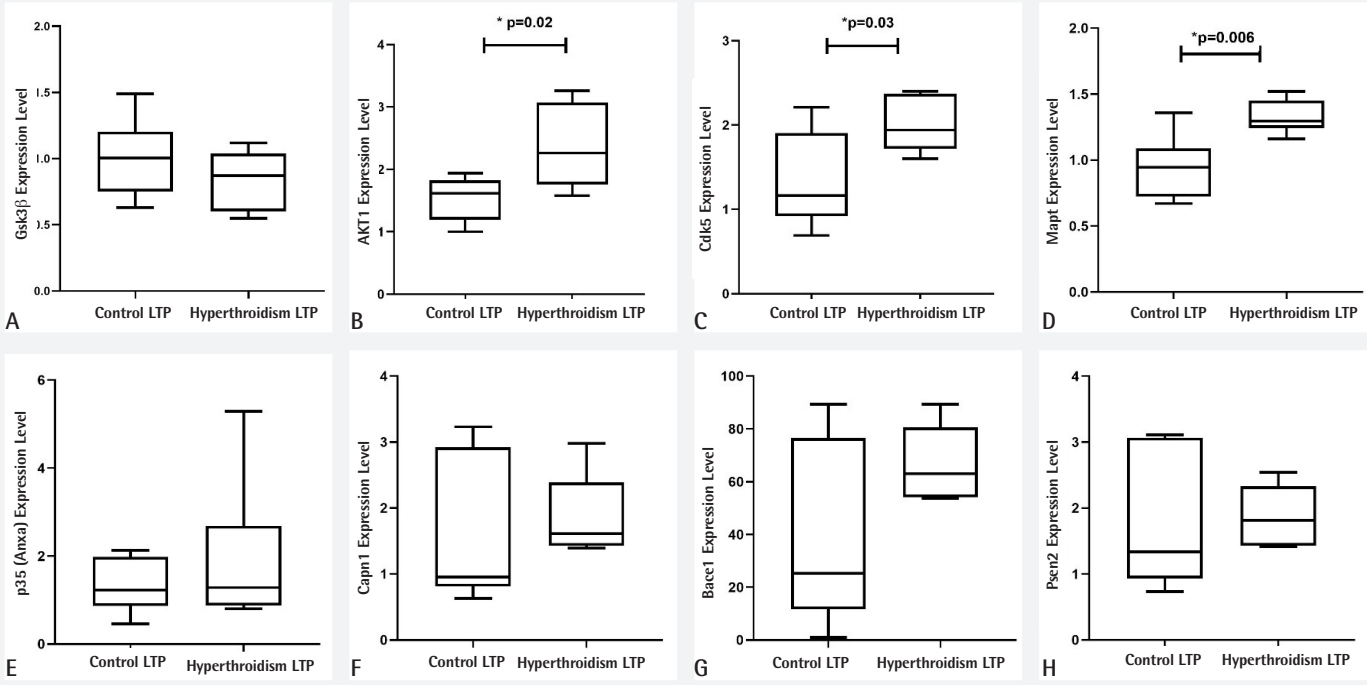

Figure 4. A-F) mRNA expression levels of tau protein- and amyloid precursor protein-related genes (n=6). Data are expressed as median (1st quartile - 3rd quartile)

**Indicates a significant difference compared to the control group*

LTP: Long-term potentiation

DISCUSSION

Changes in LTP and Gene Expression in Thyroid Hormone Excess

Tetanic stimulation with a frequency of one hundred Hz and a duration of 1 second can be applied as a series or as four repetitive sequences as in our study. Powerful four-repeat induction protocols are often preferred because they cause increased transcription factors and gene expression, and thus have greater potential for longer persistence of LTP. In our study, the fact that both the EPSP slope and PS amplitude were significantly higher in the control (euthyroid) group 1 hour after the application of the induction protocol indicates that this protocol is sufficient to induce a permanent LTP (Figures 2, 3). However, this protocol, which was able to induce LTP lasting at least 1 hour in rats in the control group, was able to induce a non-permanent synaptic LTP in the hyperthyroid group. There was an increase in PS amplitude 1 hour after induction in both control and hyperthyroid groups. These findings, which are consistent with the previous findings of our group[7,15,16], can be interpreted as the effect of hyperthyroidism is limited to the synapse level.

While mRNA expression levels of *Gsk3β*, *P35 (Anxa), Capn1, Bace1* and *Psen2* genes evaluated in the hippocampus tissue after LTP recording did not differ significantly between the groups, Akt1, Cdk5 and Mapt-mRNA expression levels were increased in the hyperthyroidism group compared to the control group.

The absence of a difference between the groups in the mRNA levels of *Gsk3β*, *P35 (Anxa), Calpn1, Bace1* and *Psen2* genes in hyperthyroid rats that did not show a permanent synaptic LTP 1 hour after HFS indicates that the products of these genes cannot be responsible for the deterioration in LTP.

Gsk3β is an important kinase that exhibits persistent activity under basal conditions and is inhibited by phosphorylation from the ser9 position by many kinases, including Akt[17]. Gsk3β ser9 phosphorylation, which occurs with the increased activity of the PI3K-Akt pathway during HFS, inhibits this kinase and promotes the formation of LTP, while preventing the formation of UDB[18]. In other words, the formation of LTP (with the effect of Akt1) requires Gsk3β to remain inactive, while the formation of UDB (with the activity of protein phosphatases) requires it to remain active[19].

Akt and Cdk5 proteins, which are the target products of Akt1 and Cdk5 gene expression, are proteins with strong tau kinase properties[20-23]. Serum tau levels of hyperthyroid cases are significantly increased compared to the controls[24]. Therefore, the increase in Akt1 and Cdk5 mRNA in hyperthyroid rats is consistent with the hyperthyroid condition having a higher risk of dementia than the euthyroid condition[25]. The increase

in LTP-related tau expression in the hyperthyroid condition may have a function that prevents the persistence of synaptic LTP and triggers neurodegenerative changes. The fact that Cdk5 down-regulation potentiated UDB by causing BDNF/CREB activation in hippocampal sections[26] supports the role of increased Cdk5 expression in the synaptic LTP disorder we observed in hyperthyroid rats.

Cdk5 is a neuron-specific kinase that plays important roles in many cellular functions and is mainly involved in many neurodevelopmental processes such as synaptic plasticity, learning, and memory[27]. For Cdk5 activation, it requires the binding of p35, p39, or p25 (a proteolytic part of p35) to Cdk5[28]. In Alzheimer's disease, p35, which is Cdk5 activator protein, undergoes N-terminal cleavage by calpain (calcium-dependent protease) to form p25. p25 forms a stable complex with Cdk5, which retains its active state longer, leading to hyperphosphorylation of tau. p25-bound Cdk5 also acts as a priming kinase and promotes Gsk3β-mediated Tau phosphorylation[23]. Although the monomeric form of Cdk5 is enzymatically inactive, it has been reported that it may cause an increase in Cdk5 tau phosphorylation and neurodegeneration in Alzheimer's disease[29]. Cdk5 inhibitors have been shown to protect hippocampal neurons against both abnormal tau phosphorylation and neuronal death[27]. In addition, some study findings show that Cdk5 activity facilitates LTP by increasing the number of NMDA receptors NR2A and NR2B[30]. Therefore, apart from the expression level of Cdk5, how the effect of thyroid hormones will change the activity of this kinase should also be investigated.

β-secretase, known as Bace1, is one of the first enzymes involved in the APP cleaving, and this is a neurotoxic abnormal cleaving. At the end of this cleaving, amyloid beta peptide production occurs and contributes to the development of AH[31]. However, it has been reported that Bace1 deficiency impairs the formation of LTP by causing a decrease in synaptic proteins (mGluR1 and postsynaptic PSD-95 proteins). In other words, it has been reported that inhibition or deficiency of Bace1 causes a decrease in glutamate (Glu) release with a decrease in the distribution of synaptic vesicles to the active site, and thus a weaker activation of NMDA and AMPA receptors on the postsynaptic membranes of a small number of glutamate, thereby impairing the formation of LTP[32]. On the other hand, it has been reported that Gsk3β activation increases Bace1 gene expression and increases amyloid peptide formation[17]. In our study, while there was no significant difference in Gsk3β-mRNA expression level between the control group and the hyperthyroidism group, there was no difference between both *Bace1* and *Psen2* genes.

Study Limitations

The following limitations should be taken into account when interpreting the results of our study, which examined the changes in the expression of genes related to neurodegeneration after LTP in the hippocampus of hyperthyroid rats: compared to the control group, measurement of plasma free T4 level in the L-thyroxine group as higher confirms that hyperthyroidism has occurred. Although changes in free T3 and TSH levels, consistent with the change in T4 level, are expected, plasma fT3 and TSH levels could not be measured (due to limited financial support). The real-time quantitative PCR method used in the study only gives information about the mRNA levels of the measured proteins. It does not provide information about the loss, destruction or activity of protein that may occur while being synthesized. For this reason, there is a need for further studies in which protein analyses will be performed.

CONCLUSION

Physiological levels of thyroid hormones are important for the expression of some genes accompanying the induction of plasticity to remain within physiological limits. Changes in hormone level can alter mRNA expression of genes related to neuronal plasticity, depending on the induction protocol.

Ethics

Ethics Committee Approval: The study was approved of Erciyes University Animal Experiments Ethics Committee dated 17.07.2019 and numbered 19/133.

Informed Consent: This is an animal experiment study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.A., Concept: M.A., N.D., C.S., Design: M.A., N.D., C.S., Data Collection or Processing: M.A., Analysis or Interpretation: M.A., N.D., C.S., Literature Search: .A., N.D., C.S., Writing: M.A., N.D., C.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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