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Effects of growth hormone-releasing hormone on sleep and brain interstitial fluid amyloid- β in an APP transgenic mouse model

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Abstract

Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by impairment of cognitive function, extracellular amyloid plaques, intracellular neurofibrillary tangles, and synaptic and neuronal loss. There is substantial evidence that the aggregation of amyloid β (A β) in the brain plays a key role in the pathogenesis of AD and that A β aggregation is a concentration dependent process. Recently, it was found that A β levels in the brain interstitial fluid (ISF) are regulated by the sleep-wake cycle in both humans and mice; ISF A β is higher during wakefulness and lower during sleep. Intracerebroventricular infusion of orexin increased wakefulness and ISF A β levels, and chronic sleep deprivation significantly increased A β plaque formation in amyloid precursor protein transgenic (APP) mice. Growth hormone-releasing hormone (GHRH) is a well-documented sleep regulatory substance which promotes non-rapid eye movement sleep. GHRHR^{lit/lit} mice that lack functional GHRH receptor have shorter sleep duration and longer wakefulness during light periods. The current study was undertaken to determine whether manipulating sleep by interfering with GHRH signaling affects brain ISF A β levels in APP^{swe}/PS1 E9 (PS1APP) transgenic mice that overexpress mutant forms of *APP* and *PSEN1* that cause autosomal dominant AD. We found that intraperitoneal injection of GHRH at dark onset increased sleep and decreased ISF A β and that delivery of a GHRH antagonist *via* reverse-microdialysis

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suppressed sleep and increased ISF A β . The diurnal fluctuation of ISF A β in PS1APP/GHRHR^{lit/lit} mice was significantly smaller than that in PS1APP/GHRHR^{lit/+} mice. However despite decreased sleep in GHRHR deficient mice, this was not associated with an increase in A β accumulation later in life. One of several possibilities for the finding is the fact that GHRHR deficient mice have GHRH-dependent but sleep-independent factors which protect against A β deposition.

Keywords

Alzheimer's disease; Amyloid- β ; growth hormone-releasing hormone; sleep

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the impairment of memory and other cognitive functions. There is substantial evidence indicating that amyloid- β (A β) plays an essential role in the development of AD (Hardy and Selkoe, 2002; Lemere and Masliah, 2010). The accumulation of extracellular amyloid plaques is one of the pathological hallmarks of AD (Holtzman et al., 2011). A β is produced in the CNS at highest levels by neurons and released into the brain interstitial fluid (ISF). The concentration of local ISF A β predicts the onset and amount of amyloid plaque deposition in a brain region and altering its concentration over weeks to months predicts increases or decreases in plaque deposition (Yan et al., 2009; Bero et al., 2011). Therefore, understanding the dynamics of ISF A β is important for understanding AD pathogenesis.

How ISF A β is regulated under physiological conditions is not fully understood. The release of A β from neurons is synaptic activity dependent (Cirrito et al., 2005; Cirrito et al., 2008; Bero et al., 2011). Before the accumulation of A β plaques, brain ISF A β levels fluctuate with the sleep-wake cycle in a pattern in which ISF A β concentration is higher during wakefulness and lower during sleep in two different amyloid precursor protein (APP) transgenic mouse models Tg2576 and PS1APP as well as in wild-type mice (Kang et al., 2009; Roh et al., 2012). Chronic sleep deprivation increases A β deposition in both Tg2576 and PS1APP mice (Kang et al., 2009). However, sleep deprivation involves stress which is known to increase A β pathology (Dong et al., 2004; Wilson et al., 2005; Kang et al., 2007). Therefore, manipulating sleep acutely and chronically using a non-stressful strategy will be useful to further establish the relationship between sleep and A β . One finding suggesting that the amount of sleep is directly relevant to ISF A β levels and ultimately A β deposition is that administration of the dual orexin receptor antagonist which increases sleep, decreased A β deposition in PS1APP Tg mice (Kang et al., 2009).

Sleep-regulatory substances (SRSs) are endogenous molecules that are produced in response to previous sleep-wake history and impact sleep or wakefulness to maintain sleep homeostasis (Obal and Krueger, 2003; Krueger et al., 2008). Growth hormone-releasing hormone (GHRH) is one of the well-studied SRSs which are involved in the regulation of non-rapid eye movement sleep (NREMS) duration and slow wave activity during NREMS. Specifically, systemic or central administration of GHRH enhances NREMS while inhibition of endogenous GHRH using GHRH antibodies or antagonists suppresses spontaneous NREMS (Obal et al., 1991; Obal et al., 1992; Obal and Krueger, 2004).

Chronic disruption of GHRH mechanisms in several mutant animal models resulted in disturbed sleep (Obal and Krueger, 2004). The hypothalamus, where GHRH and GHRH receptor (GHRHR) are predominantly expressed, is the site of action of GHRH-mediated NREMS duration and slow wave activity (Toppila et al., 1996; Zhang et al., 1998; Zhang et al., 1999). Functional GHRH receptive neurons are also present in the cerebral cortex (Liao et al., 2010) and mediate the GHRH induced enhancement of slow wave activity locally (Szentirmai et al., 2007; Liao et al., 2010).

The current study aimed to determine whether manipulating sleep acutely or chronically through the GHRH pathway affects brain ISF A β as well as A β deposition. We found that acute systemic administration of GHRH at the dark onset enhanced NREMS and decreased ISF A β . Blockage of endogenous GHRH using a GHRH antagonist reduced NREMS and increased ISF A β . In an APP transgenic mouse model, chronic loss of functional GHRH receptor resulted in decreased spontaneous NREMS during the light period and a corresponding decrease in fluctuation of ISF A β but no change in absolute level of ISF A β . Finally, we assessed whether chronic loss of functional GHRH receptor affected both amyloid plaque load and the amount of insoluble A β in 7 months old PS1APP mice. Despite decreased sleep in GHRHR deficient mice, their A β plaque load and insoluble A β levels in the brain were similar to that in the mice with functional GHRHR.

2. Material and methods

2.1 Animals

APPswe/PS1 E9 (PS1APP) mice overexpressing a chimeric mouse/human APP695 Swedish gene and human PSEN1 with an exon 9 deletion on a B6C3 background (Jankowsky et al., 2004) were used in this study. The GHRHR^{lit/lit} mouse on C57BL/6J background (Jackson Labs, stock number 000533) has a spontaneous point mutation in the GHRHR gene, resulting in a loss of receptor function (Godfrey et al., 1993; Lin et al., 1993). Expressing one copy of functional GHRHR in GHRHR^{lit/+} mice restores most of the phenotypes. PS1APP/GHRHR^{+/+} male mice were crossed with GHRHR^{lit/lit} female mice to obtain PS1APP/GHRHR^{lit/+} males as breeders. Then the PS1APP/GHRHR^{lit/+} males were crossed with GHRHR^{lit/lit} females to obtain PS1APP/GHRHR^{lit/+} and PS1APP/GHRHR^{lit/lit} littermates. All the animals were kept at 25°C on a 12 hr light/dark cycle. All experimental protocols were approved by the Animal Studies Committee at Washington University.

2.2 Microdialysis and electroencephalography (EEG)/electromyography (EMG) recording

At the age of 3 months, mice were implanted a guide cannula for microdialysis in the left hippocampus (bregma – 3.1 mm, 2.5 mm lateral to midline, 1.2 mm below the dura at a 12° angle) (Bero et al., 2011). An EEG electrode was placed over the right parietal bone (bregma –3.0mm and 2.5mm lateral to midline) and an EMG electrode was placed in the nuchal muscles. In addition, a common reference EEG electrode was placed in the midline over the cerebellum (Kang et al., 2009). The mice were then placed in their home cages and given 10 days to recover from the surgery.

On the day microdialysis began, a microdialysis probe (2 mm; 38 kDa molecular weight cutoff; BR-style, BioAnalytical Systems) was inserted into the left hippocampus through the

guide cannula and connected to a syringe pump (Stoelting) and artificial cerebrospinal fluid, pH 7.35, containing (in mM) 1.3 CaCl₂, 1.2 MgSO₄, 3 KCl, 0.4 KH₂PO₄, 25 NaHCO₃, and 122 NaCl was continuously perfused through the microdialysis probe at a constant flow rate of 1 µl/min (Bero et al., 2011). The EEG and EMG signals were connected to a P511K A.C. Pre-amplifier (Grass-Telefactor Instruments), digitized with a DigiData 1440A Data Acquisition System (Molecular Devices), and recorded digitally using pClamp 10.2 (Molecular Devices). After 3 d of habituation, recording was started simultaneously with microdialysis sample collection and continued for about 2 days until the end of the treatment. (Kang et al., 2009). The ISF was accessed for A β using sandwich ELISA (detail described in section 2.3 and 2.4). EEG/EMG records were scored by using SleepSign (Kissei Comtec Co., LTD., Japan) into 10-sec epochs as wakefulness, REMS, and NREMS.

2.3 Experiment 1: Effects of Acute administration of GHRH on sleep and ISF A β

For GHRH injection, the baseline EEG/EMG data and ISF A β were assessed for 24 hr before the injection. On the experimental day, a single dose of GHRH peptide (Bachem) was intraperitoneally (*i.p.*) injected into 3 months old PS1APP/GHRHR^{lit/+} males at 5 µg/kg body weight (Obal et al., 2003) at the beginning of dark onset. PS1APP/GHRHR^{lit/lit} males were used as a negative control. The microdialysis perfusion buffer was artificial cerebrospinal fluid containing 4% BSA (Sigma). The ISF A β levels were assessed with a sandwich ELISA using anti-A β ₃₅₋₄₀ HJ2 as the capture antibody and anti-A β ₁₃₋₁₈ HJ5.1-biotin as the detecting antibody (Bero et al., 2011). For data analysis, the mean ISF A β and sleep in the dark period on the baseline day and GHRH administration day were compared using paired t-test. Data were expressed as mean $\pm$ S.E.M.

2.4 Experiment 2: Acute administration of GHRH antagonist into PS1APP mice

For acute GHRH antagonist administration, the baseline EEG and ISF A β was collected for 24 hr. The microdialysis perfusion buffer was artificial cerebrospinal fluid containing 0.15% BSA (Research Products International). On the experimental day, 10 µM GHRH antagonist [Phenylacetyl-(D-Arg^{2,28},p-chloro-Phe⁶,Homoarg^{9,29},Tyr(Me)¹⁰,Abu¹⁵,Nle²⁷)-GRF (1-29) amide; Bachem] (Varga et al., 1999) was infused at 1 µl/min in the brain *via* reverse-microdialysis into 3 month old PS1APP/GHRHR^{+/+} males 6 hr after light onset. The EEG/EMG and ISF data were collected for another 6 hr after GHRH antagonist administration. The negative control consisted of continuous infusion of microdialysis perfusion buffer. ISF A β was measured with a sandwich ELISA using anti-A β ₁₃₋₂₈ m266 (a gift from Eli Lilly and Company) as the coating antibody and anti-A β ₁₋₅ 3D6-biotin (a gift from Eli Lilly and Company) as the detecting antibody (Castellano et al., 2011). For data analysis, paired t-tests were performed to compare averaged ISF A β or sleep on the same periods of baseline day and GHRH antagonist administration day. Data were expressed as mean $\pm$ S.E.M.

2.5 Experiment 3: Effects of chronic GHRHR deficiency on sleep and brain ISF A β

Spontaneous sleep, ISF A β , lactate and glucose in PS1APP/GHRHR^{lit/+} mice and PS1APP/GHRHR^{lit/lit} mice were recorded for 24 hr. The ISF lactate and glucose were measured using YSI 2700 SELECT Biochemistry Analyzer (YSI Incorporated). The ISF A β , lactate

and glucose levels were normalized to % of the mean concentration of each individual mouse during the dark period. Two-way ANOVA with repeated measures followed by Bonferroni post-tests were performed to compare the mean ISF A β , lactate, glucose, total sleep/wake duration (% of recording time), sleep/wake bout number and sleep/wake bout duration in PS1APP/GHRHR^{lit/+} mice and PS1APP/GHRHR^{lit/lit} mice during light and dark periods. Dark-light cycle and genotype were the two factors in the two-way ANOVA. The differences of mean daily absolute concentrations of ISF A β , lactate and glucose between PS1APP/GHRHR^{lit/+} mice and PS1APP/GHRHR^{lit/lit} mice were compared using Student's t-test. Data were expressed as mean $\pm$ S.E.M.

2.6 Experiment 4: Effects of chronic GHRHR deficiency on brain A β deposition

PS1APP/GHRHR^{lit/+} mice and PS1APP/GHRHR^{lit/lit} mice were aged to 7 months. On the day of harvesting, mice were perfused with ice-cold PBS containing 0.3% heparin. One hemibrain was fixed in 4% paraformaldehyde for immunohistochemistry. The other hemibrain was dissected and flash-frozen on dry ice and then stored at -80°C for biochemical assays.

For histological experiments, serial coronal sections at 50- μm thickness were collected from the rostral to the caudal end of each brain hemisphere using a freezing sliding microtome (Leica). A β plaques were immunostained using in-house generated biotinylated anti-A β_{1-13} monoclonal antibody HJ3.4B (Tran et al., 2011; Liao et al., 2014). For fibrillar plaques, brain sections were stained with 0.025% Thioflavin S (Sigma). Immunostained brain sections were scanned using a Nanozoomer slide scanner (Hamamatsu Photonics).

Quantitative analysis of immunopositive staining was performed as previously described (Kim et al., 2012). Briefly, images of immunostained sections were exported with NDP viewer (Hamamatsu Photonics), converted to 8-bit grayscale using ACDSee Pro 2 software (ACD Systems) and threshold was set to highlight positive staining and analyzed using ImageJ (National Institutes of Health). 3 sections per mouse (Bregma, -1.4 mm caudal to Bregma, -2.0 mm caudal to Bregma) were quantified (the hippocampus and the cortex immediately dorsal to the hippocampus) and the mean area covered by A β or thioflavin S in these sections was used to represent each mouse. Two-tailed Student's t-test was used to determine if there were significant differences between genotypes within same gender. Data were expressed as mean $\pm$ S.E.M.

For biochemical assessment of A β levels in PS1APP/GHRHR^{lit/+} mice and PS1APP/GHRHR^{lit/lit} mice, brain cortices were sequentially homogenized with cold PBS (soluble A β fraction) and then 5 M guanidine buffer (insoluble A β fraction) in the presence of 1X protease inhibitor mixture (Roche). The levels of A β were measured by sandwich ELISA. For A β_{40} or A β_{42} , anti-A β_{35-40} HJ2 or anti-A β_{37-42} HJ7.4 were used as capture antibodies, and anti-A β_{13-18} HJ5.1-biotin (Bero et al., 2011) was used as detecting antibody. These antibodies were produced in-house and are described in the references (Koenigsknecht-Talboo et al., 2008; Bero et al., 2011). Two-tailed Student's t-test was used to determine if there were significant differences between genotypes within same gender. Data were expressed as mean $\pm$ S.E.M.

3. Results

3.1 Acute administration of GHRH increased NREMS and decreased ISF A β

To examine the effects of GHRH acute administration on sleep and ISF A β , we injected (*i.p.*) GHRH into 3 month old PS1APP/GHRHR^{lit/+} male mice (n=8) at the beginning of dark onset. These mice have a functional GHRH signaling pathway. GHRH significantly enhanced NREMS ($p<0.001$, Fig. 1A) and reduced wakefulness duration ($p<0.001$, Fig. 1B) and ISF A β ($p<0.05$, Fig 1C) during the dark period following GHRH administration. REMS was not significantly affected. However, the sleep-wake status and ISF A β (Fig. 1D, E, F) were not changed in the negative control PS1APP/GHRHR^{lit/lit} mice (n=9) lacking functional GHRHR.

3.2 Acute blockade of endogenous GHRH signaling decreased sleep and increased ISF A β

To determine the effects of endogenous GHRH on sleep and ISF A β , we acutely administered a GHRH antagonist to 3 month old PS1APP/GHRHR^{+/+} mice *via* reverse-microdialysis and recorded their sleep and hourly ISF A β (Fig. 2A, C, E). The results show that GHRH antagonist significantly reduced NREMS ($p<0.01$, n=7, Fig. 2B) and increased wakefulness ($p<0.01$, n=7, Fig. 2D) as compared to the same period during the baseline day. Also, ISF A β increased with GHRH antagonist treatment ($p<0.05$, n=11, Fig. 2F).

3.3 Effects of chronic GHRHR deficiency on sleep, brain ISF A β , lactate and glucose

To evaluate the effects of chronic loss of GHRHR function, the spontaneous sleep (Fig. 3A, C), ISF A β , lactate and glucose over a 24 hr period were compared in 3 month old PS1APP/GHRHR^{lit/lit} and PS1APP/GHRHR^{lit/+} (control) males. NREMS and wakefulness during the dark period were similar in PS1APP/GHRHR^{lit/lit} and PS1APP/GHRHR^{lit/+} mice (n=9/group, Fig. 3B, D). During the light period, PS1APP/GHRHR^{lit/lit} mice had significantly less NREMS ($p<0.01$, Fig. 3B) and longer wakefulness ($p<0.01$, Fig. 3D) as compared to PS1APP/GHRHR^{lit/+} mice which possess functional GHRHR. The NREMS bout number in PS1APP/GHRHR^{lit/lit} mice was similar to that in PS1APP/GHRHR^{lit/+} mice while the NREMS bout duration in PS1APP/GHRHR^{lit/lit} mice was significantly shorter than that in PS1APP/GHRHR^{lit/+} mice ($p<0.001$ during light period and $p<0.05$ during dark period, Supplementary Fig. 1). ISF A β (Fig. 3E), lactate (Fig. 3H) and glucose (Fig. 3K) levels were normalized to the mean of those values during the dark period when the sleep is same in both genotypes. ISF A β (Fig. 3F) in both PS1APP/GHRHR^{lit/+} (n=9, $p<0.001$) and PS1APP/GHRHR^{lit/lit} (n=11, $p<0.001$) mice significantly fluctuated with light-dark cycle. However, the mean light period ISF A β (as a % of the dark period) in PS1APP/GHRHR^{lit/lit} mice was significantly higher than that of PS1APP/GHRHR^{lit/+} mice ($p<0.01$, Fig. 3F), suggesting a diminished diurnal fluctuation of ISF A β in PS1APP/GHRHR^{lit/lit} mice as compared to PS1APP/GHRHR^{lit/+} control mice. Interestingly, the mean ISF A β absolute concentrations over 24 hr are similar in PS1APP/GHRHR^{lit/lit} mice and PS1APP/GHRHR^{lit/+} mice ($p>0.05$, Fig. 3G).

The hourly ISF glucose and lactate were also assessed in PS1APP/GHRHR^{lit/+} mice (n=7) and PS1APP/GHRHR^{lit/lit} mice (n=8). In mice expressing GHRHR (PS1APP/GHRHR^{lit/+}) ISF lactate fluctuated with light-dark cycle. ISF lactate was ~20% lower during light when

the animals sleep longer than during dark period ($p < 0.001$, Fig. 3I). In PS1APP/GHRHR^{lit/lit} mice, the mean daily absolute concentrations of ISF lactate were similar to that in PS1APP/GHRHR^{lit/+} mice ($p > 0.05$, Fig. 3J). However, the fluctuation of lactate in PS1APP/GHRHR^{lit/lit} mice was diminished because lactate during light period was higher in these mice than that in PS1APP/GHRHR^{lit/+} mice ($p < 0.01$, Fig. 3I). While the lactate levels fluctuated with dark-light cycle, ISF glucose in both PS1APP/GHRHR^{lit/+} and PS1APP/GHRHR^{lit/lit} mice was stable across the dark and light period ($p > 0.05$, Fig. 3L). In addition, the average daily ISF glucose absolute concentrations in PS1APP/GHRHR^{lit/+} and PS1APP/GHRHR^{lit/lit} mice were similar ($p > 0.05$, Fig. 3M).

3.4 Effects of chronic GHRHR deficiency on A β pathology in 7 month old PS1APP mice

To determine whether chronic decreased sleep due to GHRHR deficiency influences the amount of A β deposition, we assessed A β plaque load as well as soluble and insoluble A β levels in the brain cortices in the PS1APP/GHRHR^{lit/+} mice and PS1APP/GHRHR^{lit/lit} mice at 7 months of age. The body weight of these PS1APP/GHRHR^{lit/lit} mice (15.5 ± 1.8 grams for females, 19.3 ± 4.2 grams for males, mean $\pm$ SD, $n=10\sim11$ /group) was about 50% – 60% of that seen in the PS1APP/GHRHR^{lit/+} mice (26.1 ± 5.4 grams for females and 36.2 ± 5.0 grams for males, mean $\pm$ SD, $n=10\sim11$ /group). We found that although females had more A β plaque load (Fig. 4) and fibrillar plaques (Fig. 5) than males in both genotypes, A β plaque load in hippocampus and cerebral cortex were similar in 7 month old PS1APP/GHRHR^{lit/+} and PS1APP/GHRHR^{lit/lit} mice (Fig. 4, 5). In the cerebral cortex, the insoluble A β_{40} and A β_{42} levels were similar in PS1APP/GHRHR^{lit/+} mice and PS1APP/GHRHR^{lit/lit} mice (Fig. 6B). However, the PBS-soluble A β_{40} and A β_{42} levels in the female PS1APP/GHRHR^{lit/lit} mice were significantly lower than that in female PS1APP/GHRHR^{lit/+} mice (Fig. 6A). In male PS1APP/GHRHR^{lit/+} and PS1APP/GHRHR^{lit/lit} mice, cortical PBS-soluble A β levels were similar (Fig. 6A).

4. Discussion

The current study was undertaken to determine whether brain ISF A β and A β deposition are affected when the sleep is manipulated acutely or chronically through a NREMS promoting SRS - GHRH. Our data show that when NREMS was increased by acute GHRH administration, ISF A β concurrently decreased. On the other hand, inhibiting NREMS using a GHRH antagonist resulted in an increase of ISF A β . PS1APP/GHRHR^{lit/lit} mice which have non-functional GHRHR had relatively higher ISF A β and lactate during the light period versus the dark period, when they had less NREMS than the PS1APP/GHRHR^{lit/+} control mice due to the significantly shorter NREMS bout duration. However, A β deposition at the age of 7 months was similar in PS1APP/GHRHR^{lit/lit} compared to that in PS1APP/GHRHR^{lit/+} control mice. Overall, the data suggest that in contrast to substances that increase wakefulness and increase ISF A β such as orexin, molecules that increase sleep such as GHRH decrease ISF A β . This is further evidence that acute modulation of the sleep-wake cycle dynamically modulate ISF A β .

It has been observed in mice and humans that ISF A β levels are higher during wakefulness and lower during sleep under physiological conditions (Kang et al., 2009; Roh et al., 2012). ISF A β increased with sleep deprivation and decreased during the following recovery sleep

(Kang et al., 2009). Orexin increased wakefulness and ISF A β (Kang et al., 2009). A dual orexin receptor antagonist administered for 24 hours suppressed ISF A β levels and abolished the diurnal variation of A β (Kang et al., 2009). The current study found that inducing sleep with GHRH decreased ISF A β and suppressing sleep using a GHRH antagonist increased A β . GHRH and the GHRH antagonist can act on the hypothalamus to change the global sleep/wake state of the animal. GHRH and GHRHR are present in the cortex but have not been assessed with current techniques in the hippocampus. While it is possible effects of GHRH and its receptor are local, the global effect of GHRH on the sleep/wake cycle should affect ISF A β in many brain regions including the hippocampus. Overall, these data provide further evidence that the diurnal fluctuation of ISF A β is associated with sleep-wake stage rather than the time of the day.

The mechanism underlying the sleep-wake associated change of ISF A β is not completely understood yet. It has been reported that sleep improves the clearance of exogenous A β that is injected into the brain tissue (Xie et al., 2013). On the other hand, the release of ISF A β by neurons is affected by sleep-wake state. It has been shown that A β release is up-regulated by neuronal activity both pre- and post-synaptically (Cirrito et al., 2003; Cirrito et al., 2005; Cirrito et al., 2008). Our lab observed that diurnal fluctuation of ISF A β is closely associated with ISF lactate (Bero et al., 2011), a marker reflecting neuronal activity (Pellerin and Magistretti, 1994; Uehara et al., 2008). This strongly suggests that A β release is regulated by neuronal activity that is higher during wakefulness and lower during sleep that contributes to the diurnal fluctuation of A β (Bero et al., 2011). The current study is consistent with the previous observation in that a relative increase of wakefulness during light period in PS1APP/GHRHR^{lit/lit} mice is accompanied by elevated ISF A β and lactate. The differences in ISF A β and lactate cannot be attributed to REMS because REMS duration in PS1APP/GHRHR^{lit/lit} mice was not different from that in control mice. Interestingly, although we observed a diurnal fluctuation of ISF lactate aligning with previous studies (Bero et al., 2011; Dash et al., 2012), the ISF glucose levels in the current study were relatively stable across the 24 hr cycle. Glucose level increased during NREMS in rat frontal cortex as detected by a sensitive methods with high temporal resolution (Dash et al., 2013). In mouse frontal cortex, glucose increases at the beginning of NREMS followed by a decline as a NREMS episode continues (Naylor et al., 2012). An earlier study reported that the glucose during NREMS is only 12% higher than during wakefulness (Netchiporouk et al., 2001). Given the small difference of glucose levels between NREMS and wakefulness and the fact that mice are polyphasic sleepers which have minute to minute changes in the sleep-wake cycle, our hourly assay of ISF glucose does not provide enough temporal resolution to detect the changes of glucose associated with sleep-wake stages. Besides the differences between methods, the animal species and the brain regions might also account for the different observations among different studies.

Our previous work showed that chronic sleep deprivation increased A β plaque load in APP transgenic mouse models (Kang et al., 2009). However, in the current study, we did not observe a change in A β plaque load or insoluble A β level in GHRHR deficient PS1APP mice as compared to normal PS1APP mice although they have ~90 min less of NREMS per day than the control mice expressing one copy of GHRHR. Interestingly, while we found that the diurnal fluctuation of ISF A β was diminished in the PS1APP/GHRHR^{lit/lit} mice due

to higher relative A β levels during the light period, the 24 hr absolute mean concentration of ISF A β in 3 month old PS1APP/GHRHR^{lit/lit} was only slightly but not significantly higher from that in PS1APP/GHRHR^{lit/+} mice at 3 months of age. In addition, 7 months old PS1APP/GHRHR^{lit/lit} females even had lower A β in soluble brain lysates although the insoluble A β levels (that present in the A β plaques) were similar to that in PS1APP/GHRHR^{lit/+} mice. Since GHRHR deficiency enhances cellular stress resistance, delays the aging process, and extends lifespan (Liang et al., 2003; Sun et al., 2013), it is possible that PS1APP/GHRHR^{lit/lit} mice have some sleep-independent beneficial factors which help in some way in enhancing clearance or inhibit overall production of soluble A β , therefore counteracting the effects on A β accumulation caused by less sleep. A recent study in a 5XFAD Tg mouse model demonstrated that chronic GHRH antagonist administration improved cognitive performance and decreased expression of several genes related to A β generation such as APP, BACE2 and presenilin (Jaszberenyi et al., 2012). The decrease in soluble A β in the PS1APP/GHRHR^{lit/lit} female mice observed by our lab could be explained by this mechanism although we don't know why the effect is more potent in females than males. Another potential explanation could be that the lower insulin-like growth factor-I (IGF-1) in GHRHR^{lit/lit} mice (Donahue and Beamer, 1993) decreased A β accumulation due to reduced IGF-1 signaling. Reduced IGF-1 signaling has been shown to be protective against both cognitive changes and A β aggregation in APP Tg mice (Cohen et al., 2009). In addition, the delayed developmental and aging process in the GHRHR^{lit/lit} mice might also in some way delay plaque deposition counteracting the effects of sleep loss. Finally, it is also possible that the effect of decreased sleep on plaque load may require decreasing NREMS to a greater extent than occurred in GHRHR^{lit/lit} mice. In future studies, more work is needed to better understand the relationship between sleep, aging and Alzheimer's disease.

5. Conclusions

Our data support the hypothesis that diurnal fluctuation of ISF A β is associated with sleep-wake behavior. In the PS1APP mouse model, acute GHRH administration enhanced NREMS and decreased ISF A β . Acute blockade of endogenous GHRH signaling resulted in a reduced NREMS and elevated ISF A β . Chronic loss of GHRH signaling led to a diminished diurnal ISF A β fluctuation due to decreased NREMS and increased ISF A β during light period. However, chronic spontaneous sleep loss in GHRHR deficient mice did not exacerbate A β accumulation possibly due to the fact that GHRHR deficient mice possess certain intrinsic sleep-independent factors which are protective against age-related A β accumulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Effects of acute GHRH administration on sleep and ISF A β . After 24 hr baseline collection, three month old PS1APP/GHRHR^{lit/+} and PS1APP/GHRHR^{lit/lit} males were injected (*i.p.*) with GHRH (5 μ g/kg body weight) at the beginning of dark onset (n=8–9/group). Hourly NREMS (A), wakefulness (B) and ISF A β (C) in PS1APP/GHRHR^{lit/+} mice were measured. The bar graphs represent the mean values of NREMS, wakefulness and ISF A β during dark period on the GHRH treatment day and baseline day (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; paired t-test). (D, E, F) Hourly NREMS, wakefulness, and ISF A β in PS1APP/GHRHR^{lit/lit} mice are shown on the baseline day and GHRH treatment day. The mean values during the dark period on the baseline day and GHRH treatment day (bar graph) were compared using paired t-test. Data are expressed as mean $\pm$ S.E.M.

Fig. 2.

Effects of acute GHRH antagonist administration on sleep and ISF A β . After 24 hr of baseline recording for EEG and ISF A β , 10 μ M GHRH antagonist was infused into the brains of 3 months old PS1APP/GHRHR^{+/+} males *via* reverse-microdialysis at 6 hr after light onset. (A, C, E) Hourly NREMS, wakefulness and ISF A β during a 12 hr period on baseline day and treatment day. (B, D, F) Mean NREMS, wakefulness and ISF A β before (hr 1–6) and after (hr 7–12) GHRH antagonist infusion during the light period on baseline day and administration day were compared using paired t-test (*, $p < 0.05$; **, $p < 0.01$; $n = 7 \sim 11$). Data are expressed as mean $\pm$ S.E.M.

Fig. 3. Effects of chronic GHRHR deficiency on sleep, ISF A β , lactate and glucose. Three month old PS1APP/GHRHR^{lit/+} and PS1APP/GHRHR^{lit/lit} males (n=7~11/group) were monitored for spontaneous sleep (A), wakefulness (C), ISF A β (E), ISF lactate (H) and ISF glucose (K) every hour for a 24 hr period. The mean NREMS (B), wakefulness (D), ISF A β (F), ISF lactate (I) and ISF glucose (L) during light or dark period was compared between PS1APP/GHRHR^{lit/+} and PS1APP/GHRHR^{lit/lit} mice using two-way ANOVA with repeated measures followed by Bonferroni post-test (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). (G, J, M) Mean 24 hr absolute concentrations of ISF A β , lactate and glucose at 1 μ l/min flow rate. Data are expressed as mean $\pm$ S.E.M.

Fig. 4.

Effects of chronic GHRHR deficiency on Aβ plaque load in cerebral cortex and hippocampus. PS1APP/GHRHR^{lit/+} mice (11 females, 10 males) and PS1APP/GHRHR^{lit/lit} mice (11 females, 11 males) were assessed at the age of 7 months. Brain sections were stained for Aβ plaques using the anti-Aβ antibody HJ3.4. (A) Representative images for Aβ staining in PS1APP/GHRHR^{lit/+} and PS1APP/GHRHR^{lit/lit} males and females are shown (scale bar = 1 mm). (B) Quantification of % area covered by Aβ plaques in cerebral cortex (top) and hippocampus (bottom). Data are expressed as mean ± S.E.M.

Fig. 5.

Effects of chronic GHRHR deficiency on fibrillar plaque load in cerebral cortex and hippocampus. PS1APP/GHRHR^{lit/+} mice (11 females, 10 males) and PS1APP/GHRHR^{lit/lit} mice (11 females, 11 males) were assessed at 7 months of age. Brain sections were stained for fibrillar plaques using Thioflavin S. (A) Representative images of Thioflavin S staining in PS1APP/GHRHR^{lit/+} and PS1APP/GHRHR^{lit/lit} males and females (scale bar = 1 mm). (B) Quantification of % area covered by fibrillar plaques in cerebral cortex (top) and hippocampus (bottom). Data are expressed as mean $\pm$ S.E.M.

Fig. 6. Effects of chronic GHRHR deficiency on soluble and insoluble Aβ levels in cerebral cortex. PS1APP/GHRHR^{lit/+} mice (9 females, 10 males) and PS1APP/GHRHR^{lit/lit} mice (10 females, 9 males) were assessed at the age of 7 months. Brain cortices were sequentially homogenized in cold PBS and 5 M guanidine. (A) Aβ₄₀ and Aβ₄₂ levels in the PBS fraction of cortical lysates (*, $p<0.05$; **, $p<0.01$, Student's t-test). (B) Aβ₄₀ and Aβ₄₂ levels in the guanidine fraction of cortical lysates. Data are expressed as mean $\pm$ S.E.M.