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Review Article

The role of the GABAergic system on insomnia

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Abstract

Sleep is an essential activity for the survival of mammals. Good sleep quality helps promote the performance of daily functions. In contrast, insufficient sleep reduces the efficiency of daily activities, causes various chronic diseases like Alzheimer's disease, and increases the risk of having accidents. The GABAergic system is the primary inhibitory neurotransmitter system in the central nervous system. It transits the gamma-aminobutyric acid (GABA) neurotransmitter via $GABA_A$ and $GABA_B$ receptors to counterbalance excitatory neurotransmitters, such as glutamate, noradrenaline, serotonin, acetylcholine, orexin, and dopamine, which release and increase arousal activities during sleep. Several studies emphasized that dysfunction of the GABAergic system is related to insomnia, the most prevalent sleep-related disorder. The GABAergic system comprises the GABA neurotransmitter, GABA receptors, GABA synthesis, and degradation. Many studies have demonstrated that GABA levels correlate with sleep quality, suggesting that modulating the GABAergic system may be a promising therapeutic approach for insomnia. In this article, we highlight the significance of sleep, the classification and pathology of insomnia, and the impact of the GABAergic system changes on sleep. In addition, we also review the medications that target the GABAergic systems for insomnia, including benzodiazepines (BZDs), non-BZDs, barbiturates, GABA supplements, and Chinese herbal medicines.

Keywords: *Benzodiazepines, Chinese herbal medicine, Gamma-aminobutyric acid, Insomnia, Sleep*

Introduction

Sleep and its significance

Sleep is an essential biological process to maintain optimum physical and mental health [1]. Sleeping 7–9 h per night is recommended for adults [2]. Short sleeping time or lack of sleep may weaken the immune system [3], impair cognitive functions [4,5], and alter hormonal homeostasis [6]. Sleep is monophasic; a single block usually lasts 7–8 h in humans. It comprises 90-min cycles alternating between the non-rapid eye movement (NREM) period and rapid eye movement (REM) period, which are classified based on electro-oculography activity by detecting patterns of eye movement [7]. Irregular sleep patterns in humans often occur as a result of either lifestyle choices such as work shifts [8], circadian-rhythm disturbances due to jet lag [9], excessive screen time and media usage at night [10], or due to pathophysiological conditions such as insomnia, sleep-disordered breathing [11], obstructive sleep apnea [12], and neurodegenerative disorders such as Alzheimer's disease and cancers [13]. The financial impact of decreased productivity due to sleep loss is immense; according to the study of Hafner *et al*., in 2017, the United States lost an estimated $411 billion US dollars annually [14]. Hence, it is essential to understand the underlying mechanisms for sleep loss and potential medications that could help offset its negative consequences.

Prevalence and symptoms of insomnia

Insomnia is a sleep disorder (SD) with difficulty falling asleep, staying asleep, and having good sleep quality [15]. It occurs in 50% of primary care patients and one in three of the adult population worldwide [16]. Clinical diagnosis of insomnia can be accessed by the complaint of difficulty falling asleep at night, awakening in the middle of the night, getting up too soon in the morning, finding it hard to get back to sleep, and having daytime tiredness or sleepiness [17]. According to these symptoms, insomnia patients have difficulties in performing their daily tasks and a high risk of exposure to accidents [18].

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Classification of insomnia

In the third Edition of the International Classification of Sleep Disorder-3, insomnia can be classified into three types according to sleep duration: short-term insomnia disorder, which happens shorter than 3 months; chronic insomnia disorder that presents sleep disturbances at least three times per week longer for 3 months, and other insomnia disorders that do not match with the criteria for the two types mentioned above [19]. Besides, insomnia can be categorized as primary or secondary (co-morbid). Primary insomnia (PI) is present without other co-existing diseases, while secondary insomnia occurs accompanied with other medical conditions, such as psychiatric disorders and drug abuse [20].

Pathophysiology of insomnia

The pathophysiological of insomnia has been well studied, and the imbalance between arousal and sleep-regulatory molecules is one of the causal factors [21]. Neurotransmitters are the chemical messengers that carry, promote, and balance signals between neurons and target cells throughout the body [22]. The arousal neurotransmitters include noradrenaline, serotonin, acetylcholine, orexin, and dopamine, while gamma-aminobutyric acid (GABA) and adenosine are sleep-inducing neurotransmitters that function in the ventrolateral preoptic (VLP) nucleus in the hypothalamus [21]. During wakefulness, the ascending activity sent from nuclei in the brainstem and posterior hypothalamus stimulates cholinergic neurons, monoaminergic cell bundles, and orexin nuclei in the lateral hypothalamus, inhibiting the VLP nucleus that usually promotes sleep. In contrast, neurotransmitters GABA and adenosine in the VLP nucleus inhibit the ascending activity during sleep [23], resulting in a transition from NREM sleep to REM sleep cycles [24]. Based on the dynamic interactions of neurotransmitters, GABA appears to be an essential neurotransmitter that modulates sleep. Therefore, understanding the role of the GABAergic system on sleep is necessary for developing a better insomnia treatment.

The impact of the GABAergic system changes on sleep

The effect of gamma-aminobutyric acid levels

In addition to its role in sleep, GABA is directly or indirectly involved in normal brain functions, including cognition, memory, and learning [25,26]. It is the primary inhibitory neurotransmitter in the brain and counterbalances the excitatory neurotransmitter glutamate [27]. GABAergic neurons are primarily located in the basal forebrain and the anterior hypothalamus. They are essential in modulating sleep by releasing a high level of GABA during sleep to inhibit cells that stimulate arousal functions [28]. Previous studies have revealed that SD is associated with GABA levels [29-31]. A potassium channel $Kv1.1^{-/-}$ mouse model study demonstrated that SD exacerbates seizure and reduces GABA levels in granular cells within the dentate [31]. Subjects with sleep duration of <6 h per night had shown lower GABA levels in the anterior cingulate cortex and medial prefrontal cortex, examined by magnetic resonance spectroscopy [32]. In PI patients, the cortical GABA levels measured by proton magnetic resonance spectroscopy were 12% higher than that in healthy subjects, which negatively correlated with time awake after sleep onset [33]. In contrast, in 2008, Winkelman *et al*. reported a reduction of GABA levels by nearly 30% in the brains of patients with PI [34]. These studies suggest that alteration of GABA level is associated with PI.

Gamma-aminobutyric acid synthesis and degradation

Glutamate decarboxylase 65/67

As shown in Figure 1, GABA is synthesized in the cytoplasm of the presynaptic neurons from its precursor, glutamate, via catalysis of glutamate decarboxylase (GAD) [35]. GAD belongs to the aspartate aminotransferase family of Pyridoxal 5′-phosphate-dependent enzymes [36]. There are two isoforms of GAD, GAD65 and GAD67, encoded by the *Gad2* and *Gad1* gene, respectively [37]. GAD65 and GAD67 significantly differ in the first 100 N-terminal amino acid residues, in which GAD65 is hydrophobic while GAD67 is hydrophilic [38]. Besides, GAD65 is mainly localized at the presynaptic nerve terminals, but GAD67 is distributed throughout the cells [39]. GAD65 self-activates to carry out its enzymatic function, ensuring the rapid generation of GABA pulses in circumstances requiring swift synthesis and release. Previous research reported that GAD65 knockout mice demonstrate fatal seizures and anxiety behavior [40]. On the other hand, GAD67 was responsible for more than 90% of basal GABA synthesis [41]. GABA levels are reduced for the mice lacking GAD67, resulting in neonatal death [42]. GAD67-GFP knock-in mice after SD demonstrated typical spontaneous sleep-wake patterns compared to wild-type mice [43]. However, increasing the activity of GAD67-positive neurons in the ventral tegmental area by chemogenetics activation can regulate sleep/wakefulness, especially during NREM sleep [44]. This evidence indicates the critical role of GADs on sleep quality.

Gamma-aminobutyric acid-transaminase

The GABA shunt is the biochemical pathway responsible for the catabolism of GABA. This reaction is catalyzed through the activity of the enzyme GABA-transaminase (GABA-T), which breaks down GABA into succinic semialdehyde (SSA) and glutamate [45]. After that, released GABA from the presynaptic axon terminals was uptake to both glia and presynaptic nerve terminals, followed by degrading to SSA [Figure 1] [46]. GABA-T serves as the pivotal enzyme in GABA breakdown. Blocking this enzyme significantly raises GABA levels in the brain, which has been correlated with several pharmacological effects, such as drugs treating alcoholism [47], epilepsy [48], and Alzheimer's disease [49]. In order to better understand the role of GABA-T in sleep, further research needs to be conducted. Currently, only one study has reported that Drosophila brains lacking GABA-T could promote daily sleep and sleep consolidation [50].

Gamma-aminobutyric acid receptors

Gamma-aminobutyric $acid_A$ receptors

$GABA_A$ receptors ($GABA_ARs$) are ionotropic receptors and ligand-gated chloride channels located in the postsynaptic

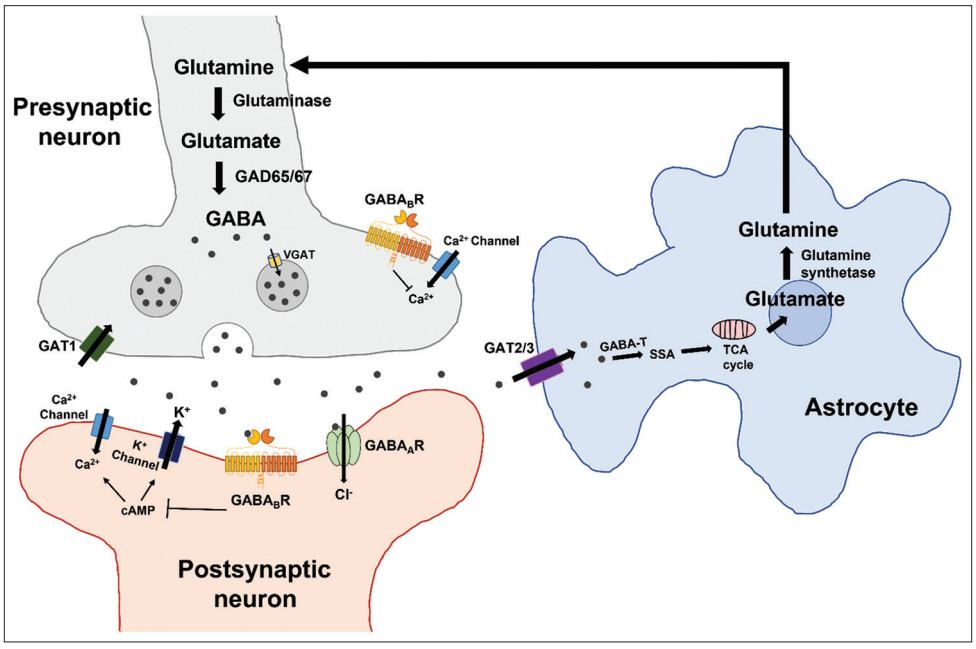

Figure 1: An illustration of gamma-aminobutyric acid (GABA) synthesis, release, uptake, and degradation in the synaptic cleft. In presynaptic neurons, glutamine is degraded to glutamate by glutaminase. Then, glutamate is converted to GABA. GABA is packed into vesicles through vesicular GABA transporter (GAT). After that, GABA is released to the synaptic cleft and binds to $GABA_A$ receptor on the postsynaptic neuron to promote chloride (Cl^-) influx or binds to $GABA_B$R on the presynaptic and postsynaptic neuron to inhibit cyclic adenosine monophosphate that controls calcium influx and potassium efflux. GABA is also uptake to its presynaptic cleft by GAT1 or astrocyte by GAT2/3. GABA in the astrocyte is degraded into succinic semialdehyde and glutamate by the enzyme GABA-transaminase. Then, glutamine synthetase converts glutamate to glutamine. Subsequently, glutamine is released and uptake to the presynaptic neuron. GABA: Gamma-aminobutyric acid, VGAT: Vesicular GABA transporter, GAT 1: GABA transporter 1, $GABA_A$R: $GABA_A$ receptor, GABA-T: GABA-transaminase, SSA: Succinic semialdehyde

sites to mediate fast inhibitory effect [51]. $GABA_A$Rs are widely presented in the brain, especially the hippocampus, hypothalamus, and cerebral cortex [52]. The structural features of $GABA_A$Rs are heteropentamers composed of 19 subunits. However, only some subunits have been identified as significant for sleep modulation, including the alpha subunits (α1–α5), beta subunits (β1–β3), gamma subunits (γ1–γ2), delta, epsilon, and the theta subunits [53]. In insomnia patients, the increased age was related to reduced mRNA levels of $GABA_A$R α1 and α2 subunits in peripheral blood, which resulted in poor sleep quality and shortened sleep time [54]. In mice with SD, the $GABA_A$Rs expression on the membrane of orexin neurons in the hypothalamus was more remarkable than in controls [55]. Besides, evidence indicates that loss of $GABA_A$R α3 subunits on thalamic reticular nucleus neurons promotes delta wave activity during sleep in mice [56]. A $GABA_A$R β1-subunit systemic knockout mouse strain demonstrated abnormal sleep phenotype accompanied by increased delta power in NREM sleep and reduced theta power in REM sleep [57]. Since several studies have indicated that $GABA_A$Rs play prominent roles in regulating sleep, modulating $GABA_A$Rs expression can be one of the approaches for treating insomnia.

Gamma-aminobutyric acid$_B$ receptors

$GABA_B$ receptors ($GABA_B$Rs) are G-protein coupled metabotropic receptors, functioning as dimers, and transforming neurotransmitter signals in the synapses to cellular responses by binding and activating heterotrimeric G-proteins [58,59]. $GABA_B$Rs are located in postsynaptic somatodendritic compartments and presynaptic sites in the axon terminals of excitatory neurons and inhibitory interneurons [60]. They respond to the slower and prolonged GABA-mediated inhibitory transmission by modulating calcium (Ca^{2+}) and potassium (K^+) channels through inhibiting cyclic AMP signals [Figure 1] [60,61]. The $GABA_B$R has two subunits: $GABA_B$-R1 and $GABA_B$-R2. $GABA_B$-R1 is responsible for receiving extracellular ligand-binding, while $GABA_B$-R2 is essentially engaged in the intercellular signal transduction and strengthened coupling to G-proteins [62]. In animal studies, $GABA_B$R agonists can increase slow-wave sleep while minimally impacting REM sleep. On the other hand, $GABA_B$R antagonists can decrease slow-wave sleep [63,64]. The expression level of the $GABA_B$R is decreased in para-chlorophenylalanine-induced insomnia in rats. The symptom can be ameliorated by a Chinese sedative Songyu Anshen Fang, which restored the $GABA_B$R expression levels in the hypothalamus [62]. Besides, the $GABA_B$-R1 receptor was found to be increased in the hippocampal CA1 region of mice with SD [65]. Hence, understanding the dynamic functions of $GABA_B$Rs may help develop novel approaches to treat insomnia.

GABAergic-targeting compounds for insomnia treatments

Benzodiazepines

Benzodiazepines (BZDs) are a class of sedative medication that help reduce brain activities. They have been widely used to treat insomnia since the 1970s and are still prescribed [79], including estazolam, flurazepam, temazepam, triazolam, quazepam, and lorazepam. BZDs act on BZD binding sites located between the α-and γ-subunits

Table 1: Gamma-aminobutyric acid ergic-targeting compounds for insomnia

Medicine	Mechanism of action	Effect on sleep	Dosage limit (mg), prescription drug time	Adverse effect	Reference
Benzodiazepines					
Estazolam	$GABA_AR$ agonist	Decrease sleep latency, nocturnal awakenings, and wakefulness after sleep onset Increase total sleep time	1–2 (7–10 days)	Headache, somnolence, asthenia, hypokinesia, nausea	[66]
Flurazepam	$GABA_AR$ agonist	Decrease sleep latency Increase total sleep time and sleep quality	15–30 (4 weeks)	Dizziness, drowsiness, light-headedness, and ataxia	[67]
Temazepam	$GABA_AR$ agonist	Decrease initial sleep latency and wakefulness after sleep onset Increase total sleep time	7.5–30 (7–10 days)	Rebound insomnia, anterograde amnesia, psychological dependence, anxiety	[68]
Triazolam	$GABA_AR$ agonist	Decrease sleep initiation Improved mean sleep onset and sleep maintenance	0.125–0.5 (7–10 days)	Somnolence, dizziness, a feeling of lightness, coordination problems	[69]
Quazepam	$GABA_AR$ agonist	Decrease sleep latency and total wake time	7.5–15 (7–10 days)	Daytime somnolence, drowsiness, fatigue	[70]
Lorazepam	$GABA_AR$ agonist	Increase total sleep time Decrease total wake time	2–4 (4 weeks)	Drowsiness, oversedation, weakness, impaired coordination, disorientation, confusion	[71]
Nonbenzodiazepines hypnotics					
Eszopiclone	Allosteric coupling to Benzodiazepine receptors	Decreased sleep latency and wake after sleep onset Increased total sleep time	1–2 (<1 week)	Metallic aftertaste, somnolence, myalglia	[72]
Zaleplon	$GABA_AR$ selective agonist (Benzodiazepine ω1 receptor subtype)	Improved sleep efficiency 4 h postadministration Reduced sleep latency	5–10 (2–4 weeks)	Headache, somnolence, dizziness	[73]
Zolpidem	$GABA_AR$ selective agonist (Benzodiazepine ω1 receptor subtype)	Reduced sleep fragmentation Increase in NREM sleep	5–10 (4 weeks)	Dizziness, drowsiness, nausea	[74]
Zopiclone	$GABA_AR$ agonist (a1 and a2 subunits)	Decreased sleep latency and Wake after sleep onset Increased slow-wave sleep	3.75–7.5 (4 weeks)	The metallic aftertaste, dry mouth, lightheadedness	[75]
Barbiturates					
Pentobarbital	Direct stimulation of $GABA_AR$	Increase in NREM sleep stage 2 Decrease in REM sleep onset and duration	0.15–0.20 (single use, intramuscular injection)	Restlessness, vomiting, headaches, loss of balance and coordination, addiction	[76]
Secobarbital	Direct stimulation of $GABA_AR$	Increase in total sleep time Slight decrease in REM sleep	0.10 (<1 week)	Somnolence, dizziness, nervousness	[77,78]

REM: Rapid eye movement, NREM: Non-REM, $GABA_ARs$: Gamma-aminobutyric $acid_A$ receptors

of $GABA_ARs$ to enhance GABAergic transmission [80], resulting in the increase of sleep time and decrease of sleep latency, nocturnal awakenings, and wakefulness after sleep onset [66]. Although BZDs effectively promote and maintain sleep, they produce several adverse effects [Table 1] such as drowsiness, oversedation, weakness, impaired coordination, disorientation, and confusion. Since the half-lives of most BZDs last longer than 8 h (except for triazolam), fatigue, psychomotor, and neuropsychological dysfunction have been noted [79]. Furthermore, BZDs have the same potential to be addictive as opioids and cannabis [81]. Therefore, adjusting the specific dosages of BZDs for individuals is essential to avoid risky side effects.

Nonbenzodiazepines hypnotics

Non-BZD hypnotics, also known as "Z" drugs, including Eszopiclone, Zaleplon, Zolpidem, and Zopiclone as listed in Table 1. They selectively bind to the α1 subunit of the $GABA_AR$, resulting in sedative effects [82,83]. Because of their selectivity, they result in lesser side effects like vomiting, convulsions, and tremors than BZDs; however, they may lead to side effects such as headaches, light-headedness, anxiety, hallucinations, and difficulty with coordination [84,85].

These drugs also have shorter half-lives than BZDs and are helpful for sleep induction but not the maintenance of sleep duration [86].

Barbiturates

Barbiturates as shown in Table 1 are another class of sedatives, usually used daily for insomnia treatment. However, long-term use of barbiturates may cause aversive side effects, such as agitation, confusion, drowsiness, hallucinations, and headaches [76,87]. Barbiturates influence CNS functions and produce sedative effects by acting on the alpha and beta subunits of the $GABA_AR$ [88]. The acting of barbiturates increases chloride ion influx and potentiates $GABA_ARs$ even in lower concentrations of GABA. In addition to the side effects, barbiturates are known to be addictive, thus leading to dependence and abuse, which is a higher risk than BZDs [89].

Gamma-aminobutyric acid supplements

GABA is commonly found in microorganisms, plants, and animals [90]. It is widely applied to functional food and pharmaceutical products. The study showed that daily drinking 250 mL GABA-enriched tea at concentration 181 mg/100 g before sleep can improve insomnia symptoms by enhancing sleep efficiency and reduced latency to sleep onset [91]. Another report found that the combination of GABA and L-theanine reduced sleep latency and prolonged sleep duration in the pentobarbital-induced sleep model [92]. Yamatsu *et al*. reported in 2016 that subjects receiving oral administration of 100 mg GABA, 30 min before sleep for a week, had shortened sleep latency and enhanced NREM sleep time [93]. GABA supplements are indeed effective in promoting sleep quality.

Chinese herbal medicines

Several CHMs used to treat insomnia have fewer side effects and are inexpensive and easy to obtain [94]. Many of them also contain chemicals that modulate the $GABA_AR$ [95] but there is little evidence for the $GABA_BR$ [96]. Xi Fan Lian (*Passiflora incarnata*) displayed hypnotic activity by acting as $GABA_B$ and $GABA_ARs$ antagonists [96]. Suanzaorentang (*Ziziphi spinosae*) has been used to improve sleep loss in patients and was found to mediate the expression of $GABA_ARs$ but not $GABA_BRs$ in SD rats [97]. Jiaotaiwan consists of Huanglian (*Rhizoma Coptidis*) and Rougui (*Cortex Cinnamomi*), increased the time of NREM sleep and REM sleep by enhancing GABA levels in the serum, prefrontal cortex, and brain stem of SD rats [98]. Danshen (*Salviae miltiorrhizae*) water extract could shorten sleep latency and increase sleeping time in mice by acting on BDZ binding sites of $GABA_ARs$ [99]. Gancao (*Glycyrrhiza uralensis*) and Hehuanpi (*Albizzia julibrissin*) reduced the sleep latency and increased sleep duration by regulating the $GABA_A$ in mice [100]. These studies support the benefits and potential of CHMs in modulating sleep via the GABAergic system.

Conclusion

Sleep is an essential biological activity for mammals to promote memory and maintain optimal physical and mental health. SDs like insomnia lower the quality of life in many aspects, increase accidental incidents, and are associated with various chronic diseases, including Alzheimer's disease and several types of cancers. The GABAergic system is known to be essential for maintaining good sleep quality. This review article addresses the GABAergic System's critical role in regulating the sleep period. Targeting the GABAergic system thus is a promising approach to novel drug development for treating insomnia. Prescription dosage, time, and side effects should be considered when developing good insomnia drug candidates. Traditional Chinese herbal medicines used to treat SDs are effective, natural, and have fewer side effects. Active compounds identified from these Chinese herbal medicines thus would be promising novel drug candidates for insomnia treatments.

Data availability statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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Conflicts of interest

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