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Study on the Related Mechanism of Insomnia

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Abstract: As a physiological activity, good sleep is of great significance to people's life and work. In recent years, with the advancement of science and technology and the acceleration of the pace of life, people's schedules have changed, and staying up late and going to bed late have become more and more common in big cities. Work stress, along with life stress, increases the incidence of insomnia. This rapidly rising incidence of insomnia has gradually become a social problem from affecting the life and work of individuals. Insomnia has begun to attract people's attention, and more and more scientists and doctors have begun to study the onset and treatment of insomnia. Many studies have explored the mechanisms involved in insomnia in many ways. In this review, we will explore the mechanism of insomnia and its mechanism-related signaling pathways from five aspects: immune function, oxidative stress state, inflammatory response, apoptosis, and brain-gut axis system. Through the collation of research data, it was found that the mRNA expression level of TLR-related genes decreased, which could improve insomnia in rats. Reducing the levels of SOD and nos in rats can prolong the duration of insomnia; increasing the content of cAMP in rat hypothalamus can improve sleep in rats; increasing the expression of Bcl-2 protein and decreasing Bax protein in rats can improve sleep. Reducing Fas/FasL protein in rats can improve sleep quality; the gut microbiota can affect sleep in rats.

1. Introduction

Insomnia is a common physiological and psychological disease, usually refers to patients on sleep time and (or) quality is not satisfied and affects the social function or quality of life during the day disease, common symptoms have difficulty falling asleep, even sleepless all night, easy to wake up, wake up early. According to the White Paper on Healthy Sleep of Chinese People in 2022 released by the China Sleep Research Society, three-quarters of the respondents said that they have had sleep disturbances, and some of these people with sleep disturbances will develop short-term or chronic insomnia. A survey shows that 10% to 15% of adults meet the diagnostic criteria for chronic insomnia [1] and worldwide, insomnia has brought large economic losses.

There is no clear conclusion on the pathogenesis of insomnia in modern medicine, and the most widely accepted hypotheses are the excessive awakening hypothesis and the 3P hypothesis. (1) Excessive arousal hypothesis: Studies have found that the electrical frequency of the brain in

insomnia patients is faster during the day and at night, the hypothalamic-pituitary-adrenal axis is abnormally excited, the release of inflammatory factors is increased, and the functional activity of vegetative nerves is increased, etc. This hypothesis believes that the pathogenesis is excessive arousal. (2) 3P hypothesis: "3P" refers to Predisposing factors (Predisposing). Precipitating factors (Precipitating) and Perpetuating factors. This hypothesis holds that 3P factors accumulate comprehensively and reach the threshold of insomnia, so the disease occurs. Under the premise of the overawakening hypothesis, many scientists have conducted further studies on the physiological mechanisms affecting insomnia [2]. The purpose of this paper is to discuss the mechanism of current insomnia onset and the signal pathways related to the mechanism, and further infer the signal pathways that may participate in the formation of insomnia, to lay the foundation for later research.

2. Immune Function

Modern medicine believes that the normal play of immune function can protect human health and reduce the probability of illness, and immune function also plays a essential role in the progression of disease. Studies have shown that sleep is closely related to the body's defense function [3]. Lack of sleep affects people's immune function. Studies have found that the immune cytokines IL-1, IL-6, TNF, etc. As classic indicators of immune function, when their content changes, the insomnia situation of insomnia rats is improved [4-6].

2.1 TLR signal Path

TLRs is a transmembrane protein receptor, which was found by Hashimoto in 1988 when studying the embryonic development process of fruit flies, TLRs as a natural immune response is the first line of defense against external microbial infection. TLRs primarily act as homologous dimers, recognizing different ligands from bacteria. It is able to respond by recognizing pathogen-associated pattern molecules or endogenous injury-associated pattern molecules. TLRs is present in almost all subtypes of innate immune cells. These include macrophages, neutrophils, dendritic cells (DCs), natural killer cells, mast cells, basophils, and eosinophils. Activation of TLRs can induce MyD88 (myeloid differentiation factor88), TIRAP(TIR domain containing adaptor protein), and TRIF (TIR-DO) in cytoplasm - main-containing adaptor inducing interferon-β) and trif-related adaptor molecule (TRAM) recruitment and signal propagation. Given the interaction between TLRs and TRAM, it has been found that different junction molecules can trigger two different signaling pathways (Figure 1), Myd88-dependent and TRIF dependent pathways, TAK1 can also be activated by α, β of IKK(inhibitor of nuclear factor kappa-B kinase) and NF-κB essential modulator (NEMO) Composed of IκB kinase (IKK) complex. IKKβ phosphorylates IκB kinase, and IκB binds to the NF-κB subunit, inhibiting the nuclear translocation of NF-κB and leading to its degradation. Therefore, NF-κB can be transported to the nucleus to promote the transcription of proinflammatory cytokines such as IL-6, IL-12, p40, and TNF [7]. Qiao Tie et al. showed through rat experiments that schisandra can reduce the expression level of mRNA of related genes through TLR/NF-KB signaling pathway, thus improving the insomnia of rats [8].

3. Oxidative Stress

Reactive oxygen species (ROS) are continuously produced through non-enzymatic and enzymatic reactions in the metabolic process of human body, but are continuously eliminated under the synergistic action of antioxidant enzymes and exogenous and endogenous antioxidants. Under physiological conditions, the generation and removal of ROS are in a dynamic balance and can be

maintained at a very low level of benefit and harmless. Due to the abnormal metabolism of the body caused by endogenous and or exogenous stimuli, a large number of reactive oxygen species are suddenly produced, or the body is insufficient in antioxidant substances, so that the balance between pro-oxidants and antioxidants is abnormal, so that the body is in a state of oxidative stress, which can oxidize biomolecules, and further cause cell death and tissue damage, and affect the sleep of the human body.

Oxidants in the body mainly include reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS are mainly superoxide anions (O_2 or HO_2) and hydroxyl radicals (OH) and their active derivatives such as hydrogen peroxide (H_2O_2), 'O_2 and LO-, LOO- and LOOH lipid peroxides. RNS, mainly NO and NO/O_2, NO/O- reaction of a series of nitrogen-containing compounds. Free radicals can oxidize and damage biological molecules such as DNA, lipids, and eggs. OH can attack deoxyribose, break down deoxypentose, and break the diacetate phosphate bond, causing single or double strand breaks in DNA. In the presence of O_2, the unsaturated fatty acids in the phospholipids of biofilm lipids are easily attacked by free radicals and their active derivatives, leading to the lipid peroxidation chain reaction, and LO-, LOO- produced in the process of lipid peroxidation can also produce chain initiation and chain amplification reactions. Both ROS and RNS can attack proteins, thus oxidizing and nitrating amino acids [9].

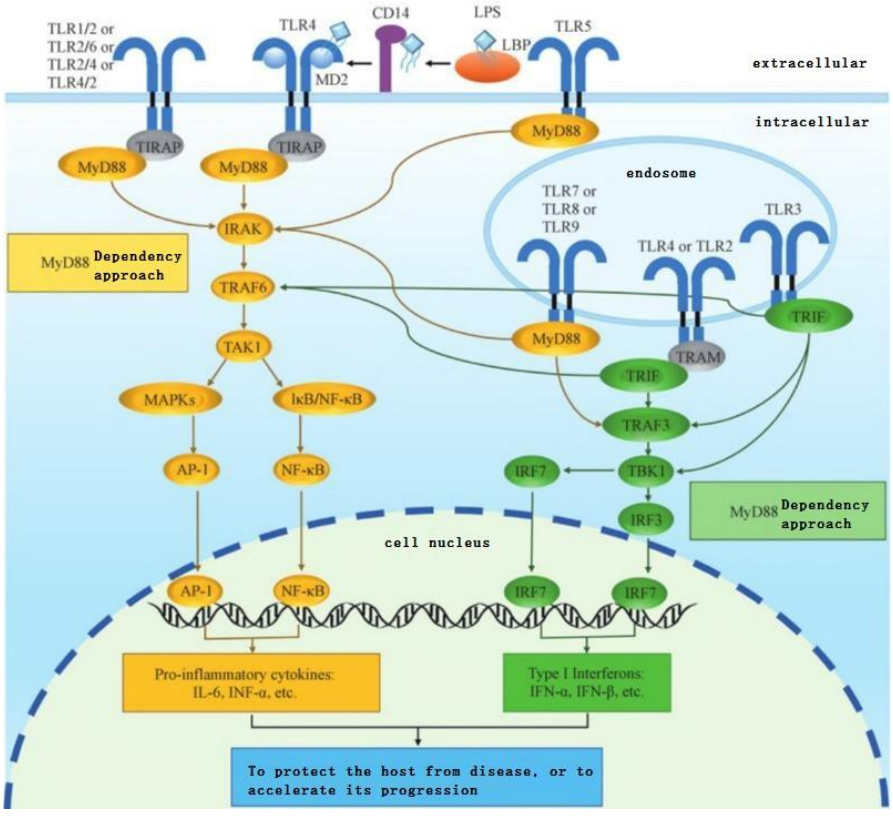

Figure 1: Typical signal path of TLRs [10]

3.1 SOD

Wang Xinyuan et al. [11] detected that the levels of CAT, GSH-Px, and SOD in the serum of insomniac patients were significantly lower than those of normal people, suggesting that the level of oxidative stress in insomnia patients was higher than that of normal people. Li Li et al. [12] showed that Wentan Decoction could reduce SOD levels and increase GSH-PX, SOD, and GSH enzyme activity in mice with insomnia, thus improving the insomnia condition of mice.

3.2 NOS

Zhao Canghuan [13] found that the contents of NO and NOS in the hypothalamus of rats in the insomnia model group were significantly higher than those in the control group. Meanwhile, it was found that wheat moxibustion could significantly shorten the sleep latency of rats with insomnia induced by pentobarbital sodium, significantly prolong the sleep duration, and reduce the contents of NO and NOS in the hypothalamus of rats with insomnia caused by PCPA. Hubaolige [14] found that moxibustion therapy in Mongolian medicine could improve the sleep status of insomnia model mice, possibly by inhibiting NOS in brain tissue and inducing excessive NO, which played a neuroprotective role.

4. Inflammatory Response

Studies have shown that chronic low-grade inflammation (CLGI) will increase the probability of insomnia. Chronic low-grade inflammation means that the level of inflammatory factors in the body is increased under physiological or environmental factors, but the infiltrating tissue is not damaged or the function is lost. In this process, the immune system continues to play a role, leaving the body in a state of non-specific and persistent chronic low-grade inflammation. Can cause a variety of neurological diseases. When an infection occurs, the body produces immune cells to eliminate the pathogen. After the pathogen is eliminated, macrophages keep the body in an anti-inflammatory state by phagocytosis of the remaining neutrophils. Subsequently, macrophages remove tissue debris and generate growth factors by phagocytosis and digestion of bacteria, viruses, fungi, or exogenous substances to protect the body from infection and injury, but if macrophages fail to effectively clear or phagocytose neutrophils, chronic low-grade inflammation will result. [15] Serological markers of inflammatory response, interleukins (IL-2, IL-6, etc.) and tumor necrosis factor-α (TNF-α), have been classic indicators in animal tests related to insomnia.

4.1 cAMP

cAMP is a second messenger in the cell, which is formed due to the activation of adenylate cyclase catalyzed by some hormones or other molecular signals. The continued transmission of the signal depends on protein kinase A (PKA). CREB, which stands for cAMP response element binding protein, is a broad-spectrum transcription factor [16]. It was found that peradenylate cyclase catalyzes the hydrolysis of adenosine triphosphate to generate cyclic adenosine phosphate (cAMP), which acts as A second messenger. cAMP further stimulates protein kinase A (PKA), which further phosphorylates CREB and downstream proteins. By regulating the function of cells, it is involved in sleep and circadian rhythm regulation [17]. In addition, cAMP also plays an important role in the inflammatory response. Cyclic nucleotide phosphodiesterase (PDE) can hydrolyze cAMP. Rolipram, a classic PDE4 specific inhibitor, can increase cAMP levels and inhibit TNF production in monocytes and macrophages. Experiments have shown that rolipram can increase the expression of MKP-1, inhibit the production of TNF in activated macrophages, and reduce the inflammatory response [18]. Zeng Aixue et al. showed through animal experiments that Songyu Anshen recipe could increase the content of cAMP in the hypothalamus of rats and increase the levels of CREBmRNA and protein phosphorylation, indicating that Songyu Anshen recipe may play a sedative and calming role by increasing the expression of GABAB receptor and regulating its mediated cAMP/ CREB signaling pathway [19].

5. Cell Apoptosis

In recent years, with the study of the association between insomnia and dementia, Parkinson's disease, depression, and other nervous system diseases, increasingly people began to pay attention to the relationship between apoptosis and insomnia.

5.1 Bcl-2 Family

Bcl-2 family is a group of proteins, which is the core molecule of the apoptosis pathway, and the most representative family member is Bcl-2 molecule. It is precisely because Bcl-2 is the most deeply studied and widely known molecule, that Bcl-2 is not only the name of the molecule, but also the name of the family. The two most prominent molecules in the Bcl-2 family are the Bcl-2 molecule and the Bax molecule. When Bax expression is high, cells are sensitive to the death signals and promote apoptosis. When Bcl-2 is highly expressed, Bcl-2 can form a heterodimer with Bax and inhibit apoptosis. Therefore, the intracellular Bcl-2/Bax ratio plays an important role in determining the sensitivity of apoptosis. Guo Jinliang et al. found through experiments that the hypnotic active component of Fructus cnidium (CHC) could inhibit the apoptosis of pineal cells in PCPA insomnia rats, possibly by increasing the expression of Bcl-2 protein and decreasing the expression of Bax protein [20, 21].

5.2 Fas/FasL

Fas (also known as CD95 or Apo1) is a death receptor through which death signals from outside the cell can be transferred into the cell. Death receptors are a class of transmembrane proteins belonging to the tumor necrosis factor receptor (TNFR) gene superfamily. There are five known death receptors TNFR-1, Fas, DR3, DR4, and DR5. The death ligand corresponding to Fas is FasL (CD95L). Hence, the Fas/FasL pathway, also known as the CD95/CD95L pathway. Fas death pathway plays an important role in the development of the immune system. Fas is expressed in many cells, but its ligand FasL is only expressed in activated T cells and NK cells. FasL is a homologous trimer, with each trimer molecule bound to three Fas molecules, and once bound to the ligand of the trimer, Fas recruits FAS-associated proteins with a death domain (FADD) in the cytoplasm through the death domain of the intracellular segment. The amino end of FADD contains a Death effector domain (DED domain), and the DED domain of FADD interacts with the DED domain of Caspase-8 molecule to recruit Caspase-8 to the Fas region. A Death-inducing signaling complex (DISC) composed of Fas, FADD, and Caspase-8 is formed. Caspase-8 is a member of the caspase family, a group of proteases that mediate efficient and specific proteolysis of dying cells and are important effector molecules in the process of apoptosis. Caspase proteolytic enzymes are in the form of zymogen and need to be activated. The activation of Caspase-8 proenzyme can activate downstream Caspase-3, Caspase-6, and Caspase-7. After activation, Casp-3, 6, and 7 can shear many substrates downstream, including ICAD (Inhibitor of caspase activated DNAase), PARP, DNA-PK, and so on. After ICAD splicing into CAD, it is transported into the nucleus, degrades chromatin DNA, and achieves cell apoptosis [21]. Xiao Hesong's experiment found that Tianwang Buxin Dan can improve the sleep quality of PCPA insomnia model rats and improve the learning and memory ability of rats, which may be achieved by reducing the expression of Fas/FasL protein [22].

6. Gut-brain Axis

The gut-brain axis (GBA) is a dynamic bidirectional neuroendocrine system. This bidirectional

communication network consists of the central nervous system (CNS), the autonomic nervous system (ANS), the enteric nervous system (ENS), and the hypothalamic-pituitary-adrenal axis system (HPA). The outer branches of the GI tract are connected to the enterobrain axis via the spinal cord and vagus nerve fibers, while the brain transmits outgoing parasympathetic and sympathetic nerves to the GI tract. Studies have found that the proportion of patients with insomnia accompanied by gastrointestinal dysfunction is the largest, and patients with gastrointestinal diseases are more likely to suffer from insomnia than healthy people, such as functional dyspepsia, gastroesophageal reflux disease and irritable bowel syndrome [23]. Ancient Chinese famous doctors also recorded in their works that "stomach disharmony leads to sleeping restlessness" and listed diseases of the spleen and stomach as one of the causes of insomnia. In modern studies, neuropeptides, which are doubly distributed in the brain and gastrointestinal tract, have been found to be involved in the regulation of sleep wake. Currently, popular sleep-related Intestinal peptides include serotonin (5-HT), Vasoactive Intestinal Peptide, and Vasoactive intestinal peptide. VIP), Glutamic Acid (GLU), and gamma-aminobutyric acid (GABA).

6.1 Gut Microbiota

The gut microbiota can affect the normal physiological activities of the brain through the neural system, endocrine factors, and related immune responses of the brain-gut axis. An experiment of intestinal microflora changes in insomnia patients in South China showed that the richness and diversity of intestinal microflora in insomnia patients decreased significantly. Modern pharmacological studies have found that intragastric administration of Jiaotai pills with water extract can improve insomnia in PCPA rats, and it has been found that after intragastric treatment, the serum HPA axis-related hormone content in rats is significantly reduced [24].

7. Conclusions

With the in-depth study of the mechanism of insomnia, researchers have found that the body's immunity, oxidative stress, inflammation, apoptosis, brain-gut axis system, and so on are inextricably related to the production of sleep disorders. A number of experimental studies have shown that the pathways related to the body's immune function, oxidative stress, inflammatory response, apoptosis, and brain-gut axis system can affect the emergence and improvement of insomnia. At the same time, apoptosis and oxidative stress are also quite complex processes, which involve a variety of proteins, molecules, and hormones. At present, only a part of proteins, molecules, and hormones have been verified to be related to sleep disorders through animal experiments, and there are still more unknown mechanisms waiting to be verified, which needs further research.

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