Studying the Role of Orexin Function and Its Effect on Stress Response

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ABSTRACT

Orexin is a neuropeptide that is produced in lateral hypothalamus neurons and sends its branches throughout the brain. These neurons play a modulating role in each region according to the main activity of the region. The two primary discovered roles of orexin include involvement in sleep processes and eating behavior. One of the important roles of orexin is the regulation of bio-neurocognitive systems related to stress response. The terminals of orexin neurons and their receptors are located in various brain areas related to memory, including the hippocampus. Stress as a homeostatic problem causes malfunction of learning and memory processes. Orexin affects the physiological and behavioral symptoms of stress by intensifying the activity of the nucleus adjacent to the ventricles of the hypothalamus and increasing the secretion of corticotropin-releasing hormone, which in turn strengthens the orexin neurons' function. This bilateral activation creates a kind of positive feedback mechanism and a stress memory form in its circuit. More studies are necessary to better understand the function of orexin in the peripheral and central nervous system, and these findings can be a valuable help for the treatment of diseases, for example, anxiety diseases. In this article, we review the findings of recent studies regarding orexin and its role in the stress system.

Key Words: *Neuropeptide, Orexin, Stress, Neurons*

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INTRODUCTION

Neuropeptides orexin A and orexin B, which are also known as hypocretin A and hypocretin B, are made from a 131 amino acid polypeptide precursor called Prepro-orexin. The gene of this peptide is located on chromosome 17 in humans, on chromosome 10 in rats, and chromosome 11 in mice [1, 2].

Orcasein-producing neurons have been conserved during evolution (from fish to mammals) and in different animals, they are mainly located in the Lateral hypothalamic area, DorsoMedial hypothalamus, and Perifornical area, with a small population (70,000 in humans and 3,000 in Rat) [3].

Prepro-orexin peptide is broken into two peptides under the effect of the ProConvertase enzyme. Orexins act through two types of receptors connected to G protein, including Orexin receptor type 1 and Orexin receptor type 2 [1]. Orexin receptor type 1 is connected to G protein-binding proteins of the 11/Gq family and is capable of activating phospholipase C and increasing the production of phosphatidyl inositol. Orexin receptor type 2 is also connected to the G protein-coupled receptor of the 11/Gq and Gi family in nerve cells **(Figures 1 and 2)** [4].

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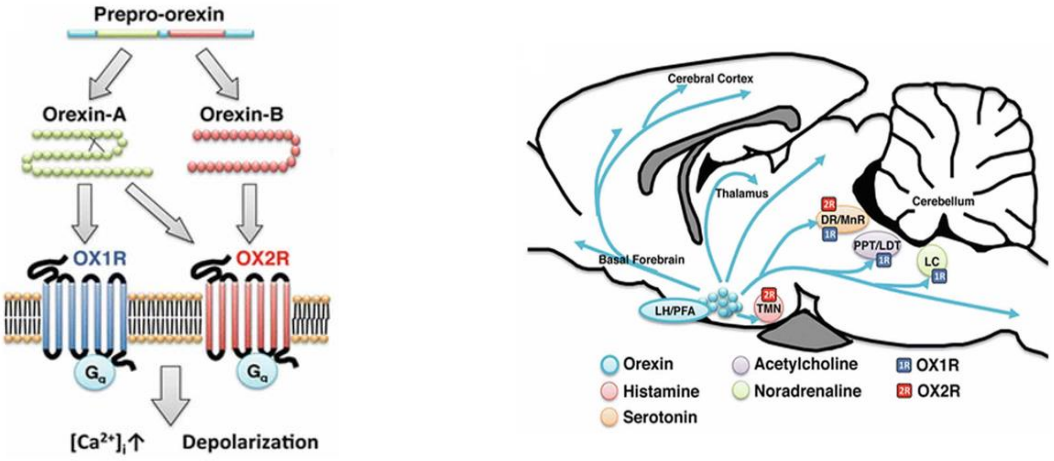

a) b)

Figure 1. How orexin is produced and effective.

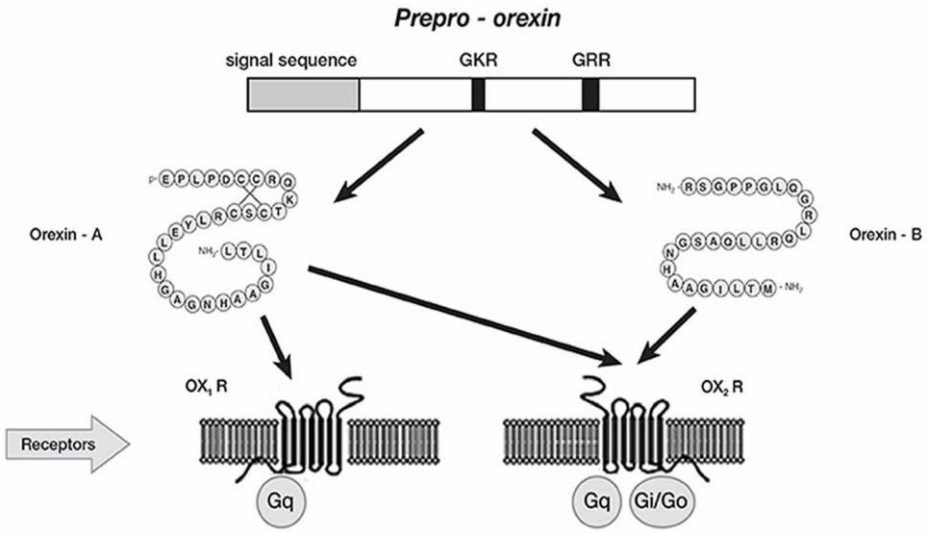

Figure 2. Orexin receptor.

Orexin neurons are located in the lateral hypothalamus and send their branches widely throughout the brain except the cerebellum [5, 6]. This wide anatomical distribution can justify the activity and influence of orexin neurons in different brain functions. Orexin neurons have dense connections with the Paraventricular nucleus, Raphe nucleus, Tuberomammillary nucleus, and especially Locus coeruleus [7]. By the extensive branching of orexin neurons, the mRNA of orexin type 1 and orexin type 2 receptors are also widely expressed throughout the central nervous system. As a result, overcasein has multiple functions, including in eating behavior, pain control, sleep and wakefulness, reward and motivation, as well as in learning and memory, epilepsy, stress, and cell death [8].

More studies are necessary to better understand the function of orexin in the central and peripheral nervous system, and these findings can be a valuable help for the treatment of diseases, for example, anxiety diseases. In this article, we review the findings of recent studies regarding orexin and its role in the stress system.

Stress

There have been many discussions about the nature of stress. Walter Canon calls stress the emergency response or fight-flight response **(Figure 3)**. Hans Selye defines stress in three levels: Alarm reaction, Resistance, and Exhaustion.

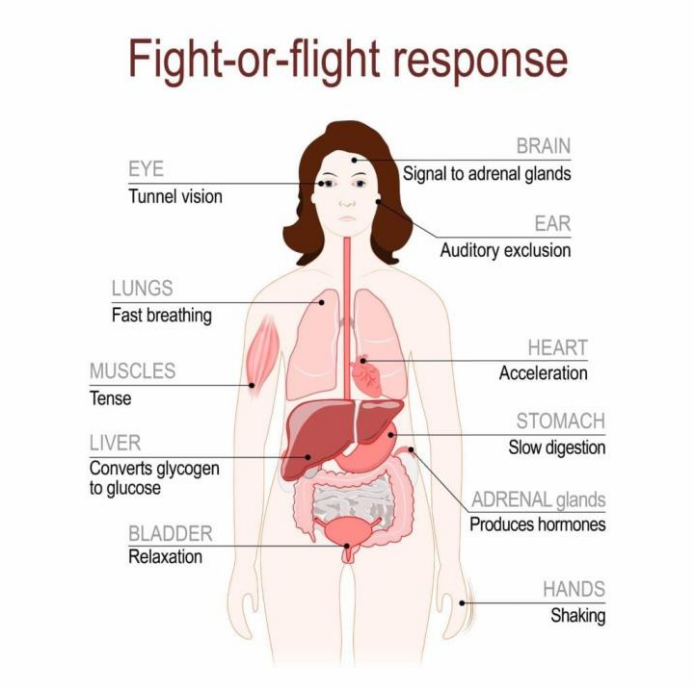

Figure 3. Fight-or-flight response.

According to the new definition, stress is a physiological or psychological stimulus that disturbs the homeostatic balance of the organism. Of course, none of the above definitions deal with the nature of stress and instead focus on the response of body systems to stress. It seems that stress is a type of physical change that leads to intense or long-term sensory input or conscious or unconscious perception, which is ultimately controlled by the cerebral cortex (such as the prefrontal cortex as the conscious and thinking part), Hippocampus (the learning and memory part of the stressful event) and Amygdala (the emotional part of a stressful event) is received and the output of these systems can follow the initial challenge, the Hypothalamus-pituitary-adrenal axis and the Autonomic nervous system as the primary systems for reacting to stress, maintaining homeostasis and restoring homeostasis activate to the initial conditions [9]. The autonomic nervous system, through sympathetic and parasympathetic nerves, creates rapid changes in response to stress in physiological states. The activation of the hypothalamus-pituitary-adrenal axis following stress causes the activation of the neurons of the nucleus adjacent to the ventricles of the hypothalamus and the release of Corticotropin-releasing hormone, which further leads to the release of Adrenocorticotropic hormone from the anterior pituitary and finally causes the production and release of the hormone glucocorticoids (including corticosterone in rats [10] and cortisol in humans [11]) **(Figure 4)**.

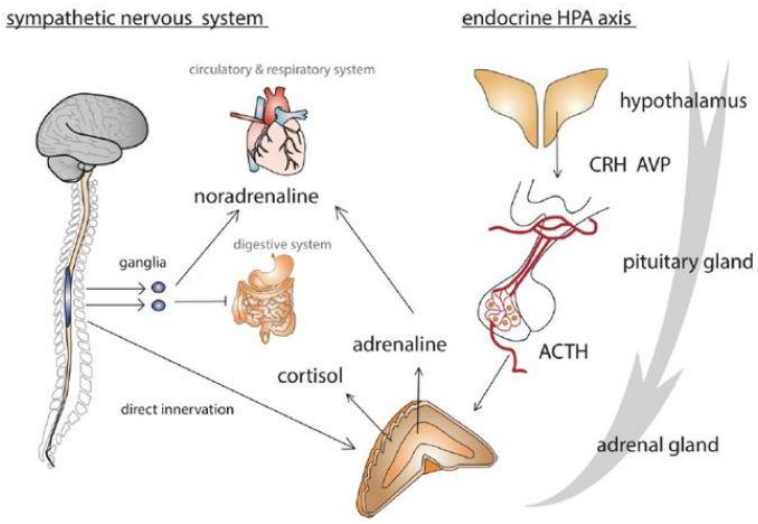

Figure 4. Human stress response pathways operate through the endocrine system and the autonomic nervous system.

The reaction speed of these two systems is such that the autonomic nervous system responds immediately, and the plasma concentration of glucocorticoids reaches its maximum value ten minutes after the onset of stress [12]. The autonomic nervous system mainly makes rapid changes in the responsiveness of body systems, while cortisol manages slower changes. In addition to these two reactive pathways, the overcasein pathway, which is known as an effective pathway in the sleep and wakefulness process, is activated by corticotropin-releasing hormone on the one hand and the amygdala on the other hand, and through the many branches of this system throughout the brain, Stress affects different parts of the brain and spinal cord and even the body [13].

Stress, lateral hypothalamus, and orexin

The lateral hypothalamus is a place for the integration of endocrine and autonomic responses and is an essential regulator of pituitary function and homeostatic balance, which regulates the stress response through the functional connection with the hypothalamus-pituitary-adrenal axis [14]. It also maintains body balance on several levels including metabolic, immune, and endocrine. Several observations support the role of orexin as a stress response modulator [15]. During stress, the activation of the hypothalamus-pituitary-adrenal axis is initiated by corticotropin-releasing hormone (which is produced in the nucleus adjacent to the ventricles of the hypothalamus). Stimulation of pituitary corticotroph cells by corticotropin-releasing hormone ultimately leads to the release of adrenocorticotropin hormone, which further stimulates the release of glucocorticoids from the adrenal gland [10]. Electron microscopy studies have shown that corticotropin-releasing hormone-containing terminals are associated with orexin-producing neurons in the lateral hypothalamus **(Figure 5)** [16].

Evidence has shown that destruction of the lateral hypothalamus has a potential role in stress. Therefore, orexin can be a very important moderator for afferents and efferents passing through the lateral hypothalamus [17]. The activity of orexin neurons is directly and dose-dependently stimulated by corticotropin-releasing hormone. In addition, Winsky-Sommerer has shown that orexin-producing neurons express the corticotropin-releasing hormone receptor on their membrane surface [13]. Studies have shown that there is a bidirectional relationship between the neurons of the ventral adjacent nucleus of the hypothalamus and the orexin neurons in the lateral hypothalamus region **(Figure 3)**. In a 2004 study by Winsky-Sommerer *et al*. it was shown that following stress and an increase in corticotropin-releasing hormone, depolarization and an increase in the firing rate of orexin neurons occur, and the membrane potential and firing rate after using corticotropin-releasing-hormone receptor type 1 returns to the basic state [13].

Anatomical observations have determined that corticotropin-releasing hormone, in addition to its direct effect on orexin neurons, also exerts excitatory effects on intermediate excitatory neurons that have synapses with orexin neurons, resulting in an increase in glutamate release from these terminals on the orexin neurons and finally, it strengthens the depolarization of orexin neurons [18]. Stress can affect the structural characteristics of orexin neurons in a sex-dependent manner. Naturally, the number of dendritic spines, especially the fungal type, is more in female orexin neurons than in males, which indicates the existence of a greater stimulating effect on female orexin neurons. Moderate repetitive stress reduces dendrite length, number of dendritic nodes, and dendritic branching in male orexin neurons [19].

Considering the many distributions of orexin to multiple brain regions and the presence of orexin receptors in different parts of the brain and body, the activation of the orexin system by corticotropin-releasing hormone during stress supports the idea that orexin can play a key role in integrating and transfer stress signals to other brain and peripheral areas. Orexin neurons send dense branching to the LC region and behavioral regions in the brainstem [5] and, for example, increase the firing rate of the subtractive neuron in the LC in vivo and in vitro [20], so the Neurotherapy system mediates the brain through these centers. Another curse is part of the stress circuit and the activation of the orexin system by stress can be part of the effects of stress. The destinations of orexin fibers are the hypothalamus-pituitary-adrenal axis, which are orexin neurons with dense branches to the adjacent nucleus sent to the hypothalamus ventricle and by affecting the type 2 orexin receptor located on neurons containing corticotropin-releasing hormone in the nucleus adjacent to the hypothalamus ventricle, it can activate neurons containing corticotropin-releasing hormone and in this way affect the activity of the hypothalamus-pituitary-adrenal axis [21]. Therefore, the signaling pathway of the corticotropin-releasing hormone type 1 receptor located on orexin neurons is an important part of the activation of the hypothalamus-pituitary-adrenal axis, and in mice where this receptor is inhibited, the activation of the hypothalamus-pituitary-adrenal axis is impaired [22]. On the other hand, inhibition of type 2 orexin receptors prevents the increase of adrenocorticotropin hormone. It can be concluded that orexin neurons and the lateral hypothalamus act as a stress signal distribution center to other areas of the brain, and such a function suggests orexin as a stress hormone along with cortisol and epinephrine. In addition, the bidirectional communication between the ventral adjacent nucleus of the hypothalamus and the lateral hypothalamic area presents a loop with a positive

feedback function that is capable of generating a type of stress memory **(Figure 5)**.

In this regard, preliminary studies have shown that intraventricular injection of orexin leads to an increase in the level of corticotropin-releasing hormone in the hypothalamus and the activation of the hypothalamus-pituitary-adrenal axis [23], and vice versa, orexin neurons are activated by corticotropin-releasing hormone [13]. In fact, in an in vitro study, increasing the membrane depolarization of neurons containing corticotropin-releasing hormone and its effect on the type 1 receptor of corticotropin-releasing hormone increased the firing rate of orexin neurons. This finding is consistent with the two-way communication between corticotropin-releasing hormone-containing neurons in the ventral adjacent nucleus of the hypothalamus and orexin in the lateral hypothalamus region and makes it possible that orexin can play a role in the hormonal response to stress [13]. It seems that in addition to the direct loop between orexin neurons and corticotropin-releasing hormone-containing neurons in the nucleus adjacent to the ventricles of the hypothalamus, there are also larger or intermediary loops.

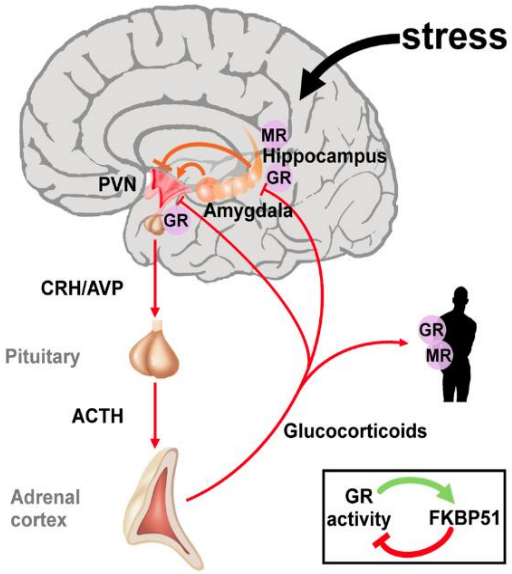

Figure 5. Neural loops intensify or weaken the activity of the hypothalamus-pituitary-adrenal axis.

Orexin and behavior

The results of the studies indicate that the orexin system is part of the circuits related to behaviors mediated by corticotropin-releasing hormone, which is activated in response to stressful situations, and in fact, the behaviors observed after orexin injection are similar to the behaviors observed during stress [17]. However, the behaviors following the injection of orexin are less anxiety-provoking than the behaviors seen after the injection of corticotropin-releasing hormone [20]. Intraventricular injection of orexin results in intense grooming with increased electroencephalographic and locomotor excitability including rearing [24], which is inhibited by orexin type 1 receptor antagonist [25]. On the other hand, the behavioral responses of mice with defects in the orexin system are reduced to emotional stress, which indicates the role of orexin-producing neurons in one of the efferent pathways of the defense response. In another experiment, it has been determined that orexin can regulate and modulate physiological responses to emotional and stressful stimuli. The cardiovascular and movement responses that healthy mice show after being exposed to aggressive mice are reduced in orexin-deficient mice [26]. These responses can also be observed during obesity and following an increase in the number and activity of orexin receptors on parasympathetic neurons located in the nucleus adjacent to the ventricles of the hypothalamus [27]. Similarly, cardiovascular responses to air flow stress in rats in which orexin was genetically deleted, decreased. These results lead to the hypothesis that orexin mediates some of the effects of corticotropin-releasing hormone in stress. According to this hypothesis, the results have shown that the activity of orexin neurons decreases during stress in mice lacking the type 1 receptor of corticotropin-releasing hormone [13].

Stress and orexin in the body

The neural network of the lateral hypothalamus is involved in many vital physiological functions in the body such as feeding behavior, sleep-wake cycle, pain inhibition, stress regulation, and reproduction. This network has a great contribution to controlling various aspects of body function because they have wide branches in the central nervous system and orexin receptors are abundant in peripheral organs [28]. Orexin receptors are expressed in the adrenal gland. Orexin type 1 and orexin type 2

receptors are expressed in the adrenal cortex, and orexin type 2 receptors are expressed in the medulla (membrane of cells that produce epinephrine and norepinephrine) [29]. Since the adrenal gland produces cortisol and epinephrine and these hormones increase orexin production by acting on orexin-producing cells, removing the adrenal gland causes a decrease in the expression of orexin precursor mRNA in the lateral hypothalamus region, which then after treatment with external glucocorticoid, it returns to the initial level. Orexin stimulates the type 2 orexin receptor in the adrenal cortex by increasing the expression of the enzymes of the steroid hormone production pathway, which increases the secretion of these hormones [30].

Applying orexin to the tissue culture medium of pig adrenal medulla increased the release of epinephrine and norepinephrine by increasing intracellular calcium, but at the same time, it decreased the expression of casein-pre-enhancer, type 1 and type 2 orexin receptor, which indicates the feedback effect. Orexin is negative in controlling the activity of the adrenal center as well as the sympathetic nervous system [31]. Branches of orexin fibers in the spinal cord showed that orexin probably plays a role in modulating sensory and autonomic input. Following stress, orexin neurons send branches to the thoracic and lumbar spinal cord and stimulate the intermediate lateral columns and also the caudal raphe [32].

Intraventricular injection of orexin or injection in specific locations in the brainstem induces sympathetic activation and the release of epinephrine and norepinephrine [33]. In particular, neural activity from the brain stem nucleus to brown fat tissue and an increase in metabolism and thermogenesis have been observed. Recently, sympathetic signaling stimulated by orexin to skeletal muscle has been reported. Intracerebroventricular injection of orexin can activate stereotyped behavior associated with stress response [34]. Other places with orexin receptors are ovaries in females and testes in males. Both orexin receptors are present in the ovary and especially in the membrane of granulosa cells, and in addition to increasing the number of gonadotropins, they also play a role in the growth and formation of follicles and increasing the number of eggs. These effects of orexin are achieved by increasing cell proliferation, changing the cell cycle, and reducing apoptosis by interfering with the AKTERK signaling pathway [35]. The effect of orexin on the orexin type 1 receptor has been traced in the membrane of testicular Leydig cells, and it shows the role of orexin in increasing testosterone production through the activation of the orexin type 1 receptor pathway [36]. On the other hand, orexin B and orexin type 2 receptors have been observed in all three regions of the epididymis (head, trunk, and tail) [37]. More importantly, orexin is produced locally in the structure of the testis and affects its receptors, and it has been observed in Sertoli cells, spermatids, spermatocytes, and seminiferous tubules [38].

CONCLUSION

The activation of the hypothalamus-pituitary-adrenal axis, the sympathetic pathway, and the release of epinephrine from the adrenal center following stress are all part of the classical pathways of stress response, which mainly act peripherally and partly centrally. Cortisol and epinephrine can affect the central nervous system and prepare for establishing homeostasis. Branches exiting from the adjacent ventral nucleus of the hypothalamus with an effect on the lateral hypothalamus region through the type 1 receptor of corticotropin-releasing hormone located on orexin neurons, is a new pathway for stress that creates a loop between the two mentioned areas and somehow leads to stress memory. becomes By activating the cores of the modulating systems and finally the amygdala, the hippocampus, and the middle prefrontal cortex, orexin is placed in higher and more complex loops, which leads to the control of stress-oriented activity. On the other hand, the presence of orexin receptors in the three mentioned areas causes functional and structural changes in these centers during acute and chronic stress and changes the quality of complex loops. Therefore, orexin can be proposed as the main possible arm of the central influence on the stress axis, which ultimately helps to control and establish homeostasis in acute stress conditions or the creation of an allostasis load in chronic stress conditions.

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