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LETTERS

Orexins: the 'multitasking' neuropeptides in the energy metabolism and immune regulation of male reproduction

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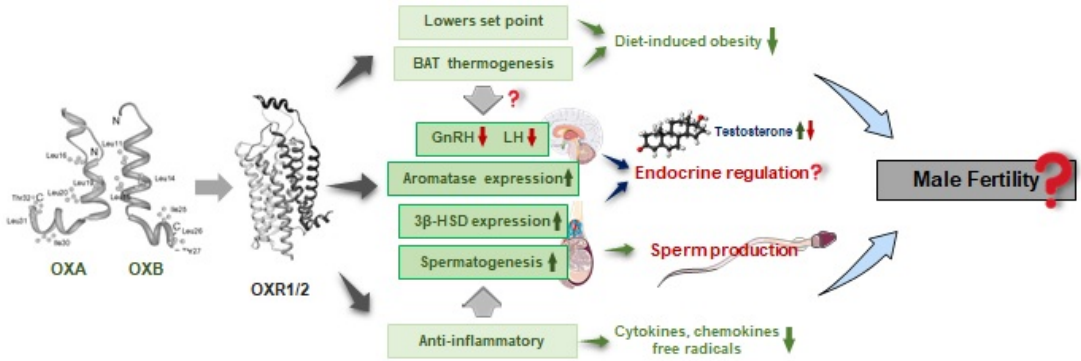
Review Article

ABSTRACT



Orexins are hypothalamus-derived neuropeptides with versatile functions. The most explored domains of orexins' functions are their influence on the central nervous system (CNS) implicated in the regulation of sleep/wakefulness cycle, food intake behavior, energy homeostasis, and cognitive processes. Orexins reportedly bear two isoforms, orexin-A (OXA) and orexin-B (OXB), which act via their specific G protein-coupled receptor (GPCR), OX1R and OX2R. These peptides also play vital roles in various other peripheral organs where they regulate metabolism, neuroendocrine functions, blood pressure, as well as reproductive functions. Interestingly orexins also exhibit immuno-regulatory, anti-inflammatory properties and facilitate the mechanism of obesity resistance. Recent research has also shed light on significant role of orexins, in particular orexin A, in regulating reproductive functions in male since the Leydig cells, Sertoli cells, germ cells in various stages of the development, and even the epididymis and penis, manifest the OXA receptor. It will be intriguing to explore the properties of the orexins in reversing obesity, downregulation of inflammatory responses and mediation of male reproductive functions. The present article thus reviews these multitudinous properties of orexins and comprehends the possible connection among the behavioral, metabolic, anti-inflammatory functions of orexins with their roles in male reproduction.

Keywords: anti-inflammatory, energy homeostasis, male reproduction obesity, orexins

INTRODUCTION

Hypothalamic neuropeptides, orexins or hypocretins (OX/HCRT) mediate a variety of physiological activities in humans and other animals. It is found in two different forms: orexin-A (OXA) and orexin-B (OXB), both of which are generated from the same prepro-orexin (PPO) precursor. Orexin receptors are also of two types, OX1R and OX2R, and mediate their functions via transmembrane G-protein coupled receptors.[1] The orexins, their receptors along with the HCRT-producing cell bodies and their projections, together is termed as the OX/HACRT system. They are profusely distributed in the hypothalamus and project to locus coeruleus (noradrenergic) and to less extent also to the thalamic regions, basal ganglia, reticular formation, nucleus of tractus solitarius (NTS), basal forebrain, amygdala, dorsal raphe nuclei, cortical regions, suprachiasmatic nucleus, olfactory bulb, cholinergic brainstem and spinal cord.[2] Orexins have significant roles the regulation of sleep-wake processes,[3, 4] emotion and cognition,[5, 6] food intake behavior,[7] and energy homeostasis.[8] Orexins' endocrine and reproductive roles have come to light in recent years, and they are mostly dependent on integrated neuroendocrine processes.[9] The activity of gonadotropin-releasing hormone (GnRH) neurons and gonadotropin-secreting pituitary cells have been found to be influenced by OXA.[10] In the control of sexual maturity and fertility, GnRH neurons are the primary integrators of internal and external signals. Different physiology related theories have

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indicated a close association of Energy homeostasis to reproductive functions. The research reports concerning increasing body weight have shown that Orexins might have a role in the control of reproductive processes as well. OX/HCRT regulates reproduction in animals by interacting with the hypothalamic-pituitary-gonadal (HPG) axis.[11, 12] Exogenous orexins' ability to modify endocrine processes has recently been matched by their prospective use in the therapy of reproductive diseases.[13]

The present article reviews the properties of orexins in reversing obesity, downregulation of inflammatory responses, and discusses the possible connection between these functions of orexins with their roles in male reproduction.

OREXINS AND OREXIN RECEPTORS

OX/HCRTs represent tightly conserved peptide product of pre-pro-orexins (PPO) which is 130 amino acids in length. This PPO gets cleaved enzymatically into two HCRT peptides: HCRT1/OXA (33 amino acid) and HCRT2/OXB (28 amino acid).[14] OXA binds to OX1R with more specificity, whereas OX2R has a similar affinity for both OXA and OXB.[1] Orexin and its receptors are found in all the mammals with considerable conservation. Both of these receptor genes are extensively expressed in the rat brain, although OX1R distribution and function differ from OX2R.[15] Apart from their distribution and activities in the central nervous system, the orexinergic system also prevails and works in numerous peripheral tissues.[16, 17]

Orexin receptors in reproductive tissues

Male reproductive functions are significantly regulated by orexins. Immunolocalization of orexin receptors in Sertoli cells, Leydig cells, resting spermatocytes, spermatogonia, round, oval, and elongated spermatids have demonstrated their importance in spermatogenesis and steroidogenesis.[18, 19]

Orexin receptors in testicular cells and epididymis

The testicular cells, epididymis, seminal vesicle, and penis all express OX1R and OX2R, but the PPO was only found in the epididymis and penis.[13, 18-21]. Orexin receptors, OXA and OX1R, have also been found in the testicular interstitium and tubular compartments during the postnatal period. OXA and OX1R expressions were discovered in gonocytes, foetal Leydig cells, and Sertoli cells on the first postpartum day, and orexins as well as their receptors were found in Leydig cells, Sertoli cells, spermatogonia, and early spermatocytes on the tenth postpartum day; Sertoli cells, spermatogonia, spermatocytes, spermatids, and Leydig cells all displayed OXA and OX1R-immunopositive signals on the thirtieth and ninetieth postpartum days, respectively. The high expression of OXA and OX1R in the testis suggest that orexins might be playing an important role in spermatogenesis and steroidogenesis, which is though yet unconfirmed.[22]

Orexin receptors expressions by the neuroendocrine regulatory components

PPO mRNA expression in the rodent hypothalamus is sexually dimorphic, with greater levels in female rats than in male rats. In contrast, male rats pituitary OX1R mRNA levels were found to be greater than female rats. In both genders, mRNAs for PPO and orexin receptors were shown to be differently expressed in peripheral organs. Furthermore, the effects of gonadal steroids 17β-estradiol and testosterone on PPO mRNA expression in female rats and orexin receptor mRNA expression in male rats were shown to be distinct. In addition, pituitary OX1Rs and adrenal OX2Rs appeared differently. These findings imply that orexin receptors play a key role in sex-specific neuroendocrine and endocrine control of reproductive processes. The hormonal profile and light-dark cycle govern significantly on orexin receptor expressions in the female reproductive tract throughout distinct reproductive cycle phases.[23] Several studies have reported the orexinergic system and its role in regulation of endocrine functions of pituitary,[24, 25] however, the mechanisms by which different hormonal environments impact the orexinergic system is unknown. PPO, OX1R, and OX2R levels in the hypothalamus and pituitary were measured in female Sprague-Dawley rats at various estrous phases, and the results were correlated with the endocrine milieu, food intake, and light-dark cycle. According to the findings, OX1R and OX2R expression rises in both the hypothalamus and pituitary during the proestrus phase but does not change during estrus or diestrus phases. Furthermore, PPO expression in the hypothalamus increased extensively during the proestrus phase.[26] During the four stages of the estrous cycle, researchers compared the expression of the PPO gene in porcine endometrium and myometrium, as well as the intensity of OXA- and OXB-immunoreactivity in endometrial glandular and luminal epithelium and stroma, as well as the myometrial longitudinal and circular muscles.[27] On days 14-16 of the estrous cycle, the endometrium and myometrium had the highest levels of PPO mRNA expression. On days 2–3 of the cycle, myometrial PPO gene expression was more prominent than in the endometrium, but endometrial gene expression was much higher in the later phase (days 17–19).[27]

PPO mRNA and OX1R were detected in various cells in the rat testis, but not OX2R,[19, 28] whereas, OX1R and OX2R mRNA expressions were detected in human testis, but not PPO mRNA.[13] OXA has been shown to enhance basal testosterone production in both in-vitro and in-vivo studies.[18, 28-30] In these investigations, low OX1R gene expression was found in Leydig cells. [13, 20, 29, 31] Orexins activate the phospholipase C (PLC) pathway via induced inositol triphosphate (IP3) synthesis, and OXB has been found to be more potent in this regard.

OREXINS AND IMMUNITY

OXA is a 'multi-tasking' molecule that regulates a number of important bodily activities. Because of its significance, the orexinergic system may be dysfunctional in a variety of clinical diseases.[32] This neuropeptide, interestingly, plays a key role in hippocampus neurogenesis, which improves spatial learning and memory. Orexin deficiency has been linked to a variety of illnesses, including depression, learning, and memory deficits.[33] OXA, like adiponectin, plays a key function in energy balance and obesity, as well as AT accumulation.[34-36] OXA is reported to alter white fat lipolysis and brown fat thermogenesis, influencing the overall energy balance.[37, 38]

Another function of OXA in inflammation has recently been discovered. OXA expression was shown to be significantly high in the stomach, lung, and kidney of rats subjected to ischemia/reperfusion.[39] OXA is implicated in immunity since inflammation is a component of the immune response.[40] Following binding to its receptors, orexins have the ability to influence both innate and acquired immunity. NF-kB activation and pro-inflammatory cytokine expression in macrophages may be suppressed by orexins.[41] Furthermore, it may be investigated whether orexins impair the responsiveness of T cells as well as the lymphopoiesis of B cells. The immune system is extremely sensitive to changes in the environment, and it is not only integrated with the autonomic nervous system but also with the central nervous system (CNS).[42] Cytokines, which are mediators of the immune system, have the ability to exert control over mediators of the central nervous system. Microglia, which are immune cells that are members of the macrophage family, are found in the central nervous system. Orexin-A/hypocretin-1 are among the neuropeptides that are involved in the development of metabolic disorders, the inflammation, and response to infection.[43, 44] Evidence on the effects of orexin-A on fat storage, food intake, and energy expenditure, as well as on the central nervous system (CNS), support the relationship between adipokines, immunity, and CNS.[41]

As narcolepsy is an immune-mediated disorder, autoantibodies may cause changes in the OXA system.[45] The loss of OXA neurotransmission in narcolepsy may be due to faulty OXA neuronal synthesis and/or secretion, or structural damage to OXA generating neurons caused by autoantibodies. Microglia and macrophages are activated during these activities, resulting in the release of neurotoxic chemicals such as quinolinic acid. These events may cause OXA neurons in the hypothalamus to be selectively destroyed.[45] In related studies, researchers used an animal model to investigate the effects of lipopolysaccharide (LPS) on the hypothalamic vigilance system and the hypothalamus production of inflammatory markers.[42, 46, 47] The hypothalamic immune response and alertness states were altered by peripheral immunological challenge with LPS.[42, 48, 49] The absence of OXA changed this reaction. Because of this, OXA plays a critical role in metabolic abnormalities, inflammation, and immunological response.

OREXINS AND ENERGY METABOLISM

The exact role of orexigenic system (orexins and their receptors) in long term energy metabolism is still not well known. Orexin neurons exhibit a unique regulatory function manifested by direct or indirect effects on different aspects of metabolism, metabolic signals control and regulate neurons activities.[1] Orexin restrictively get expressed in the lateral hypothalamic area (LHA). LHA is considered as a feeding center as it has a role in suppressing feeding behavior.[50] Recently, a considerable attention has been derived on the arcuate nucleus as a center of feeding behaviors. A correlation exists between LHA and arcuate nucleus neurons. LHA neurons function as a downstream target of arcuate nucleus orexigenic agouti-related peptide (AgRP) neurons and leptin hormone.[51, 52]

Orexin neurons exhibit an internal state enhanced by nutrients, glucose level and leptin, via their broad projections from the forebrain to the spinal cord. There are two types of orexin receptors: OX1R and OX2R which subsequently modulate the neural activities of target sites.[53] Melanin-concentrating hormone (MCH) is the first discovered orexigenic peptide, it is specifically located in the LHA.[54, 55] Later on, other feeding related neuropeptides which expressed in LHA have been described like dynorphin, galanin, and cocaine- and amphetamine-regulated transcript peptides.[56, 57]

Orexins and food intake

The most common, and firstly described physiological function related to the orexins is feeding behavior modulation. It was suggested that orexins precursors expression in the hypothalamus is increased during fasting period[58] and when orexin is centrally administered, it induces food intake which is dose-dependent.[59, 60] The feeding-promoting effect of orexin is not a robust, it tends to be similar to that initiated by galanin and MCH.[61] Although, this evidence has been obtained when orexins are administered at concentrations that are slightly higher than the usual physiological range, some research reports have also revealed that orexin system have an implication in the physiological control of feeding.[53,54] Following the central administration of anti-OXA antibodies under fasting conditions, feeding behavior inhibited in a dose dependent manner.[62]

An analytic study considering orexins and their receptors revealed that orexins signal that covers the central hypothalamic areas is involved in satiety, appetite control and regulation of energy metabolism.[63] One animal study showed that OXA increases daytime feeding, while nocturnal feeding is reduced without any change in 24-hour food intake on the second and eighth during an eight-day interval. Observations suggested that orexins may regulate feeding in certain conditions, especially when feeding pattern is influenced by circadian rhythms and during the state of hypoglycemia.[64] Another way by which orexins induce a stimulation of feeding is throughout the opioid pathway, which is best illustrated by the non-selective antagonistic effect of opioid receptors that block the orexin stimulatory action on feeding. A probable explanation is due to its effect on the reward and central pleasure mechanisms of feeding behavior.[65] During feeding process there are stomach dilation and stomach fullness, associated with the release of cholecystokinin-8 which rapidly inhibits orexins expression.[66] By altering wakefulness and feeding behaviors, orexins adapted according to the energy demands of the body, modulated the acute feeding behavior and integrated multiple metabolic signals.

In spite of orexins unequivocal role in the modulation of other physiological processes, their physiological control of food intake seems to be clear.[3, 9] In agreement with an orexin system physiological control of feeding, the orexigenic effects are mainly mediated by the neuropeptide Y (NPY) system. Interestingly, studies by immunohistochemistry had demonstrated that there are axonal synaptic interactions among orexins neurons and NPY/AgRP neurons and with NPY axons in the periventricular hypothalamus.[67] In addition, within the periventricular hypothalamus, OXA seems to modulate

spontaneous firing of glucose sensitive neurons and promote food intake throughout the NPY pathway.[68]

Orexins and glucose metabolism

Multiple studies had been done on animals about the acute effects of orexin on glucose metabolism, but the results were inconsistent. When OXA was administrated to the cerebral ventricles in rats, the level of blood glucose exhibited either an increase,[69] decreased,[70] or no change.[59] Together, OXA enhances glucose uptake by skeletal muscles by the β2-adrenergic receptor.[71] When considering the chronic effects of orexin which are represented via gene modification, orexin-deficient mice showed a mild deterioration in glucose tolerance which is age dependent and consistent while mice with an overexpressed orexin have a better glucose metabolism.[72] When a normal chow diet is used in orexin gene-modified mice, the changes in the parameters of glucose metabolism were very subtle. However, the significance of these results still undefined clearly. In contrast, when a diet rich in fat was given to them, the glucose metabolism got apparently deteriorated, this can be considered as a good model for diet induced insulin resistance as was shown in orexin overexpressed mice which showed diet induced insulin resistance.[73] In this manner, better glucose metabolism might be a consequence of a non-obese phenotype and obesity has a determinant effect in deteriorating glucose metabolism.

Orexins and energy expenditure

The orexin neuromodulatory control of energy metabolism tends to be integrated and complicated which involves several brain regions. In addition to its role in the modulation of appetite regulation, orexin also has a function in controlling energy expenditure. The long-term energy homeostasis is determined by the balance between food intake and energy expenditure.[74] A study by Lubkin and Stricker-Krongrad revealed that an increase in the basal metabolic rate (BMR) occurred after the injection of OXA into the third ventricles regions of mice brains that is irrespective to physical activity, while orexin-B failed to exhibit such an effect.[75] Thus, orexins are critical central neuropeptides for the regulation of non-exercise activity thermogenesis (NEAT). Orexins regulate brown adipose tissue (BAT) thermogenesis in a way similar to the NEAT-enhanced increase energy expenditure.[76] An increase in body temperature following the central injection of OXA in rats under anesthesia, in the absence of feeding or physical activity was also reported by Yoshimichi *et al.*[77] They stated that the capability of orexins to enhance energy expenditure is somewhat related to the increase in basal metabolic rate, non-exercise activity thermogenesis and thermoregulation, and main dominator involved in this process is OXA.[78] The neurons of orexins are highly glucose sensitive. A reduction in blood glucose level excites them and their functions are usually suppressed by excess blood glucose levels.[69] Thus, energy status can be translated into neural signals within orexin neurons.[79] During fasting (a state of low blood glucose) there is an upregulation in the orexin mRNA expression in the hypothalamus and an increase in the cAMP response element binding protein phosphorylation within orexin-responsive neurons. This supported the idea that orexin neurons are functionally responded to feeding status.[80]

An excess energy diet can stimulate orexins expression in the hypothalamus and seem to be correlated positively with the elevation in the triglyceride (TG) concentrations in the blood. So, orexin neurons are also sensitive to TG level.[81] This could explain the function of orexins in high fat diet induced eating, and subsequent development of obesity. Both increased blood levels of lipids during a positive energy balance and decreased blood glucose levels during a negative energy balance stimulate orexins system. Leptin receptor has been expressed in orexins neurons. Excess leptin may downregulate orexins level, by leptin ability to inhibit orexins expression. However, exogenous administration of leptin is usually associated with orexins upregulation.[81] Leptin is not the only polypeptide involved in orexins expression regulation. Another one; ghrelin (secreted by the hypothalamus and the stomach) is an important candidate that regulates appetite. Research findings has shown that ghrelin stimulates orexins expression via neuropeptide Y (NPY) and AgRP pathways.[82] Increased physical activity also has a stimulatory effect on orexin neurons.[83]

Orexins and obesity

High energy diet and low physical activity are the main attributable risk factors to the obesity pandemic in the modern societies nowadays. Easy accessibility to a rich fat diet leads to overweight and subsequently obesity, which provided a great prospection into the role of central hypothalamus in regulating energy metabolism.[84-87]

As mentioned earlier, orexins, sometimes termed hypocretins are originally identified as factors that enhance feeding behavior.[1] Orexin deficiency may be linked to the pathophysiology of narcolepsy.[88] In comparison with its acute effect regarding promotion of feeding, low orexin level or loss of orexin neurons resulted in obesity. It has been suggested that orexin has a considerable negative regulatory effect on energy metabolism.[40] Consistently, human adults with narcolepsy reported a higher body mass index (BMI) with an increased incidence of metabolic syndrome.[88] Among the two functional receptors of orexins, OX2R signaling is more effective in inducing resistance to diet induced obesity in some mammals.[73] The orexin receptor signaling effects on food intake and energy expenditure tend to be either acute or subacute. Limited information is available about the roles of the two orexin receptors, especially in long term control of metabolism.[89] Similar to essential orexin role in the central nervous system, peripheral effects are also relevant which are necessary for the development of brown adipose tissue (BAT), ultimately leading to alterations in metabolic phenotypes.[90]

Together with the expression of OX receptors in several brain areas, orexin neurons have a lot of processes which are related to different and multiple brain areas.[91] This distribution of orexin neurons and their receptors explains how these neurotransmitters participated in various physiological conditions and suggests an important role in obesity development.[92, 93] An important thing to be noted here is that orexin neurons exist in a baseline intrinsic state of depolarized activity[94] and largely get affected by the local lateral hypothalamic network. Activations of orexin receptors cause a state of depolarization and active neuronal firing. The

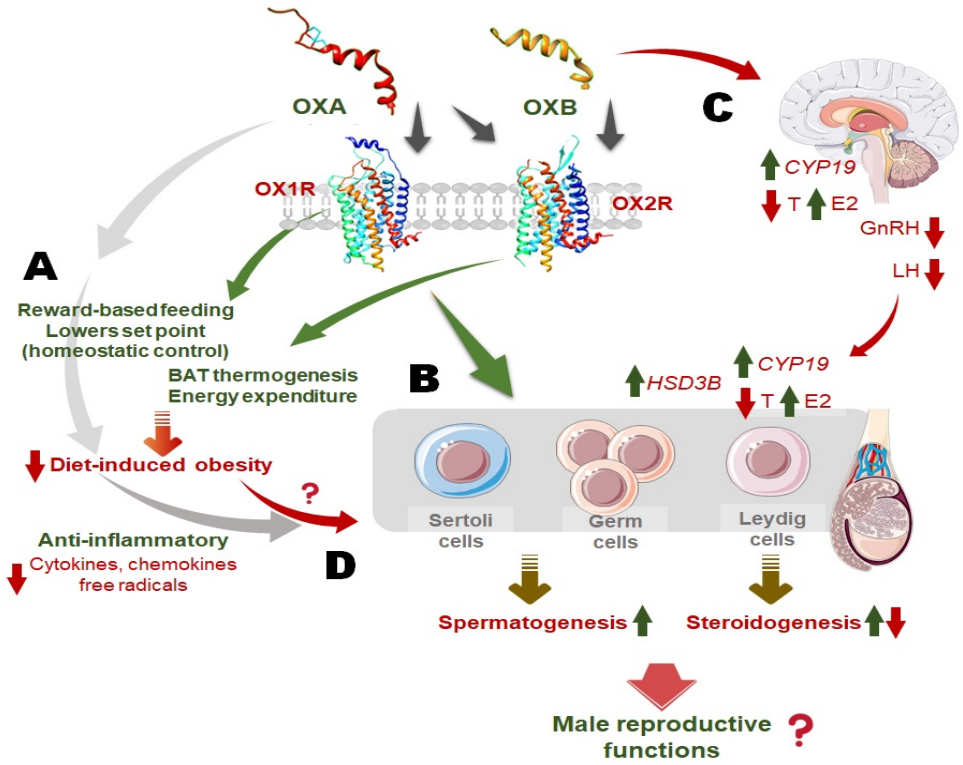

Figure 1. Mechanism of orexins-mediated energy metabolism and immune regulation in relation to male reproduction. (A) OXA through the binding of OX1R facilitates reward-based feeding and lowers set point for food intake, whereas OXB via OX2R stimulates brown adipose tissue (BAT) thermogenesis and energy expenditure. This mechanism suppresses the diet-induced obesity. OXA by acting on Sertoli cells and developing germ cells stimulates spermatogenesis, but its role on steroidogenesis is not very discrete. Via the hypothalamic and testicular aromatase expressions, OXA coverts testosterone to estrogens. It also facilitates 3b-HSD activity to stimulate testicular steroidogenesis (B-C). It has also shown anti-inflammatory responses by inhibiting the release of proinflammatory cytokines (D).

exact mechanism appears to be cell dependent via many G-proteins coupled signals end with a neuronal depolarization.[95]

Orexins on brown adipose tissue

An additional function played by OXA system has evolved due to the presence of OX receptors in multiple cerebral regions.[96] In general, experimental studies demonstrated that when orexin is injected intracerebroventricularly (ICV), food intake, locomotor activity and interscapular brown adipose tissue (IBAT) temperature (which is the most important effect of non-shivering thermogenesis) are enhanced.[97]

Different studies have exhibited that when the sensitivity to the two subtypes of orexin is increased, then this tends to get associated with an increased OX1R and OX2R expression. It is clearly evident that orexin system not only regulate the amount of body fat and body weight, but also participate in the response to other satiety-enhancing modulators such as leptin and insulin, thus it in turn contributes to the polygenic obesity despite inconsistent variation in energy intake.[98, 99] An important concept that is obviously related to obesity is energy expenditure which is positively influenced by orexin. In general, the total daily energy expenditure is defined by the summation of different components. Evaluation energy expenditure assumes a great importance because it gives us the opportunity to determine the possible nutritional and energy needs, establishment of the energy balance, understood that caloric share is necessarily maintained constant and the dimension and body composition, to support daily physical activity and ensure the long-term health outcome.[100] An important consideration in energy expenditure deserves the spontaneous physical activity. Results obtained after injections of OXA in different brain areas indicate that the levels of spontaneous physical activity are increased with a consequent increase in food intake, which might end in a state of excess body weight. Instead, as state of excess energy expenditure exerted following the activation of the orexin and this has a protective effect against obesity.[101] However, in addition to the orexins, other neurotransmitters seem to have the ability to influence spontaneous physical activity levels, for ex: leptin, cholecystokinin and corticotropin releasing hormone, but orexin is the most consistent one across all brain sites and with different types of stimulation.

Orexins in Modulation of HPG Axis

The HPG axis is the main endocrine axis for reproductive function control and is influenced by numerous additional hormone and neuronal crosstalks, for instance with thyroid,[102-104] kisspeptin,[105] melatonin,[106] other metabolic hormones.[107] The immunoreactive fibers of orexinergic system have the CNS distributions that overlap with the GnRH neurons, mainly in the arcuate nucleus-median eminence and septo-preoptic region. This suggests that orexins can influence the secretion of pituitary

luteinizing hormone (LH) by modulation of the pulsatile GnRH release.[108] Despite the fact that most research on the effect of orexins on the HPG axis employed female rodents as a laboratory model, the overall findings show that orexins have a significant influence on GnRH neurons and their secretions to control reproductive processes via changes in the HPG axis.[108] As orexins can modulate the pulse of GnRH release, they are important in controlling LH production from the anterior pituitary, which can impact testicular functioning via modulating steroidogenesis in the Leydig cells.

In estradiol benzoate and progesterone-pretreated ovariectomized rats, intracerebroventricular injection of OXA or OXB stimulated LH secretion in a dose- and time-dependent manner.[24] The effects of exogenous orexins injection, on the other hand, were shown to be dependent on the presence of ovarian steroids. Increased plasma LH levels were found in ovariectomized rats treated with 17β-estradiol and progesterone followed by orexins infusion, suggesting that estrogen upregulates orexin receptors.[109] The site-specific orexin-mediated LH response in the hypothalamus appears to be carried out by OX1R, which is found on GnRH cells.[108] As a result, orexins have the ability to increase GnRH release from the hypothalamus, although this is contingent on the presence of additional steroidal interference.

Orexinergic neurons are located mostly in the dorsomedial hypothalamic nucleus, zona incerta, lateral hypothalamus, and perifornical regions of sheep.[110] A large proportion of GnRH cells are in close contact with orexin immunoreactive terminals, indicating that orexins play a key role in GnRH neuronal regulations. Orexin receptor mRNAs have also been found in hypothalamic regions involved in neuroendocrine processes.[110]

GnRH secretion can be suppressed by orexins indirectly via -endorphins (endogenous opioid peptides).[111] Proopiomelanocortin (POMC) neurons, which are β-endorphin precursors, are innervated by orexin neurons pass via the arcuate nucleus. The effects of orexins on the mean LH concentration and the GnRH pulse frequency were reversed when naloxone, an opioid antagonist, was given with them. This finding shows that orexins are involved in reducing LH concentration and hypothalamus GnRH release pulse frequency by acting on β-endorphin.[111] In contrast to OXA, naloxone had no impact on OXB, which nevertheless greatly affected the LH level and pulse. Through its effects on hypothalamic GnRH secretion, OXB might not be dependent on β-endorphin. Because orexins control anterior pituitary response to GnRH for LH production, it is possible that gonadotrophs have a substantial proportion of orexin receptors. It has already been established that somatotrophs and corticotrophs include both orexin receptor types.[111]

Orexins and Testicular Functions

OXA is a hypothalamic neuropeptide which has a specific role in regulation of male reproductive axis and tract.[112] Parallel to central hypothalamus, OXA and its OX1R also present in peripheral organs. Earlier studies done using animal model have suggested that both OXA and OX1R located and expressed in adult mouse testis and their role in testicular development during neonatal period was also demonstrated.[113] In addition to testes, OXA and OX1R are also demonstrated and expressed in principal cells of epididymis, urethral-prostatic complex and vestibular glands of cattle. Further reports suggested that OXA had a principal role in the regulation of testicular steroidogenesis in rat.[114] However, the exact mechanism, how OXA regulates testicular steroidogenesis and the physiological significance of binding between OXA and OX1R in the testes have so far not been evaluated well. Studies revealed that there are strong immune-positive signals for OXA and OX1R mainly in the germ cells (spermatogonia, spermatocytes and elongated spermatids) supporting Sertoli cells and Leydig cells, which suggests possible involvement of both in regulation of testicular functions in adult mice.[115] OXA also seems to play a significant role in spermatogenesis control and glucose homeostasis which is best illustrated by their effect in the modulation of glucose transporter-3 (Glut3), as demonstrated using in-vitro and *ex-vivo* studies of neonatal mice.[115, 116] A role for orexins in the central control of the reproductive axis has been demonstrated recently. OXA stimulates gonadotropin releasing hormone (GnRH) release from hypothalamic explants of both male and female.[117] Similarly, hypothalamic neurons of GnRH express OX1R and receive direct signals from orexin fibers.[108, 118] OXA has the ability to either stimulate or inhibit luteinizing hormone (LH) secretion from anterior pituitary gland. This bimodal mode of action has been apparently linked with site-specific effects of OXA.[119] Some studies have tried to address the role of orexinergic system in the regulation of pituitary hormones, but the mechanism of how other hormonal milieu affects this system, is still not clear.[24, 25] Thus, orexin either acts upon the hypothalamus and pituitary to modulate the influence of hypothalamic-pituitary-gonadal (HPG) axis upon testicular functions, or acts directly throughout its receptors on testicular cells like Sertoli and Leydig cells to regulate sperm production (spermatogenesis) and testicular steroidogenesis.[112]

Orexins, Obesity and Male Reproduction

The orexin system, which is primarily concerned with increased in energy expenditure, can enhance both spontaneous physical activity and food intake when activated.[37, 120] While obesity have become pandemic in recent years, some people may be genetically predisposed to obesity. According to reports, orexins may be a crucial brain components that mediate the obesity resistance mechanism.[121, 122] Increased orexins in spontaneous physical activity have been linked to obesity resistance in animal models. Theoretically, orexin-induced spontaneous physical activity contributes to obesity resistance by increasing non-exercise activity thermogenesis.[74] However, the mechanism by which central hypothalamic orexin signalling controls spontaneous physical activity is still unknown. Obesity causes a variety of physiological problems, with one of the most serious issues towards being a disruption in normal reproductive processes.[123, 124] It is suggested that obesity bears positive association with male infertility.[125, 126] The involvement of several obesity-related hormones in control of male reproductive

processes, such as adiponectin, obestatin, ghrelin, and leptin, has been evaluated in different studies in an attempt to elucidate the mechanism by which obesity affects male fertility and semen quality.[107, 126-129]

Orexins have been shown to have a significant impact on GnRH neurons and their releases, as mentioned in earlier sections. Orexins dramatically enhance the aromatase (Cyp19) gene expression in the hypothalamus of male rats (**Figure 1**). Aromatase is an enzyme that transforms androgens to estrogen in peripheral tissues and the brain.[111, 130] Hypothalamic interneurons, such as NPY, POMC, or ghrelin, may also play a role in modulating orexin inhibitory effects on the HPG axis,[21, 131, 132] which is a complex, interconnected network affected by both central and peripheral signals. The central injection of orexins into the hypothalamus of male rats dramatically raised Cyp19 gene expression and estradiol hormone levels.[133] Orexins are neuropeptides produced in the hypothalamus that have primarily suppressive effects on the reproductive axis.[24, 134] OXA has been proven to lower LH and testosterone levels in the blood.[135] Orexins may influence GnRH and LH secretions, therefore regulating the reproductive axis.[11] It is clear from several animal studies that peptides such as orexins play a significant role in the control of testicular activities.[136]

Due to their receptor expressions in vital testicular cells, such as the Leydig cells, Sertoli cells, and spermatozoa at various developmental stages, even in the epididymis and penis, recent data have shed light on a significant role of orexins, particularly OXA, in the regulation of male reproductive functions.[13, 137, 138] It was also shown that administering rats with high levels of orexins increased testicular testosterone production.[29] For testosterone synthesis, both the adrenal glands and the gonads require four essential steroidogenic enzymes, the most significant of which is 3β-hydroxysteroid dehydrogenase (3β-HSD).[139] The expression of 3β-HSD has been found in a variety of organs[140-142] and it is also used as an immunohistochemical marker to figure out how much testosterone is produced. Orexins reportedly stimulate 3β-HSD expression in adrenocortical cells[143] and in rat primary Leydig cells[144] indicating that orexins may play a role in steroidogenesis in steroidogenic cells. The activation of the phospholipase C/inositol triphosphate cascade and the expression of functional orexin receptors in testicular peritubular myoid cells may enhance other testicular activities. Orexins have distinct effects on different parts of the male reproductive system, and the pleiotropic actions of orexin receptors might be due to numerous signalling pathways. It's been proposed that orexins impact testosterone synthesis via orexin receptors in Leydig cells, which then govern orexin receptor expression in other tissues, forming a positive feedback loop.[13]

Orexins and Male Reproductive Behavior

The lateral and dorsal hypothalamus contain the cell bodies of neurons containing HCRTs/OXs, which project to numerous regions of the brain involved in sexual behaviour. These areas mainly comprise of the medial preoptic area, the ventral tegmental area, and paraventricular nucleus.[9] In a pioneer investigation, the potential role of orexins in sexual behaviour was shown to improve sexual arousal and copulatory performance via HCRT1/OXA in the pre-optic media.[145] Consecutive investigations have shown an increase in hypocretin/orexin neuron immunoreactivity during copulation in male rats.[146] Furthermore, systemic treatment in rats of an OX1R antagonist showed that copulatory activity was reduced. It had been proposed that orexins, by stimulating the mesolimbic dopaminergic pathway in a steroid-sensitive manner, might improve the rewarding feeling of natural stimuli such as sex.[146] Specific investigations on the involvement of orexin neurons in sexual behaviour in male rats utilizing specific neural activation markers and targeted neuronal lesions have cast doubt on this theory.[147] The net findings of these research show that orexin neuron activation is increased, even when there is no additional stimulation of a receptive or non-receptive female or when there are signs of a sexual reward. During the initial mating trials, orexin neuron injuries reduced the latencies to mount and intromission. Orexins may not be necessary for male sexual performance, but they are important for sexual arousal, especially in naive animals, and they may be important for sexual reward processing.[147]

Orexins are involved in steroidogenesis, spermatogenesis, sperm transportation and maturation, and penile function regulation, including penile erection maintenance. Plenty of further research studies are required to understand the mechanisms of orexin impact on various testicular functions, [13] and, most crucially, on the impact of orexins on sperm quality, which is the most significant parameter for determining male fertility.[148-152]

Conclusion and Future Perspectives

Orexins are of great research interest owing to their diverse roles including in neuroendocrine regulation, energy balance, emotions, anti-inflammatory, and reproductive functions. Orexins may directly influence GnRH neurons and the HPG axis, thereby modulating reproductive functions. Despite evidence on expression of orexins and their receptors in male reproductive tissues, precise roles of these peptides in male fertility remain elusive. Since orexins mediate obesity resistance and possess anti-inflammatory properties, further research should be conducted to reveal whether orexins can be ameliorative in obesity or inflammation induced male infertility or subfertility.

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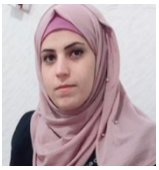

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