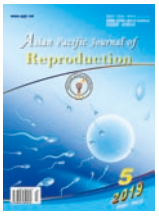

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Orexins and male reproduction

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ABSTRACT

Orexins (or hypocretins) are hypothalamic neuropeptides with a multitude of physiological functions. They occur in two known forms, namely, orexin A and orexin B with a common precursor, preproorexin. The orexin receptors (orexin 1R and orexin 2R) belong to the Family of G-protein coupled receptors. The primary function of the orexin system, *i.e.* the orexins, their receptors and associated neuronal circuitries, perhaps is to increase spontaneous physical activity and food intake, thereby promoting an increase in energy expenditure. Reports suggest that orexins may be the key brain components to mediate the mechanism of obesity resistance. Recent research also has thrown lights upon a significant role of orexins, especially orexin A, in regulation of male reproductive functions owing to their receptor expressions in vital testicular cells, such as Leydig cells, Sertoli cells as well as spermatozoa at different developmental stages, even in the epididymis and penis. Moreover, orexins have been reported to greatly influence gonadotropin-releasing hormone neurons and their secretions to regulate reproductive functions *via* modulation of the hypothalamic-pituitary-gonadal axis. Evidence thus implicates participation of orexins in steroidogenesis, spermatogenesis, transportation and maturation of sperm as well as in the control of penile function. However, further research is required in this direction to elucidate the mechanisms by which orexins play a role in different testicular functions and effect of orexins on semen quality.

1. Introduction

Orexins or hypocretins (OX/HCRT) are hypothalamic neuropeptides and mediate various physiological functions in humans and other mammals. It exists in two forms: orexin-A (OXA) and orexin B (OXB) which are derived from a common precursor, prepro-orexin (PPO). They act *via* the orexin receptors (OX1R and OX2R) which are transmembrane G-protein coupled receptors (OX2R)[1]. OX/HACRT system includes orexins, their receptors and HCRT-producing cell bodies widespread in hypothalamus, projection to the noradrenergic locus coeruleus and lesser projections to the basal ganglia, thalamic regions, the medullary reticular formation, the nucleus of the solitary tract, dorsal raphe nuclei, amygdala, cortical regions, the olfactory bulb, suprachiasmatic nucleus, basal forebrain, cholinergic brainstem and the spinal cord[2]. Orexins are well known to play a significant role in sleep-wakefulness cycle[3,4], emotion[5,6], food intake

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behavior[7], and energy metabolism[8]. In recent years, the endocrine and reproductive functions of orexins are also surfacing which mainly depends upon integrated neuroendocrine mechanisms[9]. OXA has been found to influence the activities of gonadotropin-releasing hormone (GnRH) neurons and gonadotropin-secreting pituitary cells[10]. GnRH neurons are the prime integrator of internal and external cues in regulation of sexual maturity and fertility. It has been suggested that energy homeostasis strongly associates with reproductive functions. Orexins may play additional roles in the regulation of these reproduction functions. There is a growing body of evidence indicating that OX/HCRT modulates reproduction interacting with the hypothalamic-pituitary-gonadal (HPG) axis in mammals[11,12]. Also the ability of exogenous orexins to alter endocrine functions has now been mirrored by its potential involvement in treatment of reproductive disorders[13].

This review aims to provide a clear updated concept regarding (a) the distribution of orexin receptors in reproductive tissues, (b) the influence of orexins over the HPG, and (c) the possible role of orexins in male reproductive functions.

2. Orexins and orexin receptors

OX/HCRTs are the highly conserved peptide product of PPO (130 amino acid), with two enzymatically cleaved HCRT peptides: HCRT1/OXA (33 amino acid) and HCRT2/OXB (28 amino acid)[14]. OXA binds more selectively to OX1R, while OX2R has similar affinity for both OXA and OXB[1]. Orexin structures and their receptors are suggested to be greatly conserved in all mammals. The genes for both of these receptors are found to be widely expressed throughout the rodent brain as well, but the distribution and role of OX1R differ from those of the OX2R[15] Furthermore, the orexinergic system also prevails and operates in several peripheral tissues apart from their distributions and functions in central nervous system[16,17].

3. Orexin receptors in reproductive tissues

Orexins play a significant role in the regulation of male reproductive functions. Their significant roles in spermatogenesis and steroidogenesis have been evidenced through immunolocalization of orexin receptors in the Sertoli cells, Leydig cells, resting spermatocytes, spermatogonia, round, oval and elongated spermatids[18,19]. Both OX1R and OX2R are expressed in the testicular cells, epididymis, seminal vesicle, and in the penis, while the PPO was expressed only in the epididymis and penis[13,18–21]. Studies have also confirmed the localization of orexin receptors, OXA and OX1R in both the testicular interstitium and the tubular compartments throughout the postnatal period. Studies revealed that on the first postpartum day, OXA and OX1R-expressions can be found in the gonocytes, fetal Leydig cells and Sertoli cells; on the tenth postpartum day, orexins, as well as their receptors, can be detected in the Leydig cells, Sertoli cells, spermatogonia and early spermatocytes; while on thirtieth and ninetieth postpartum days the study showed OXA and OX1R-immunopositive signals from the Sertoli cells, spermatogonia, spermatocytes, spermatids and Leydig cells. These reports indicate that owing to the considerable expressions of OXA and OX1R in the testis, orexins play a vital role in spermatogenesis and steroidogenesis, which are yet to be completely understood[22].

Sexually dimorphic expression of *PPO* mRNA has been evident in the rodent hypothalamus with higher levels in female rats than in the male rats. Whereas, the pituitary *OX1R* mRNA levels were found to be higher in male rats than in female rats. mRNAs for *PPO* and orexin receptors were found to be differentially expressed in peripheral tissues in both the genders. Moreover, it has been demonstrated that the effects of gonadal steroids, 17-β estradiol and testosterone, on mRNA expression of *PPO* in female rats and orexin receptor in male rats were different. They also differed in their actions over pituitary OX1Rs and adrenal OX2Rs. These observations suggest that the orexin receptors may have a significant role in sex-specific neuroendocrine and endocrine regulations over reproductive functions. Orexin receptors are expressed in the female reproductive tract in altered fashion according to the different reproductive cycle phases, as dictated by the hormonal profile and light-darkness cycle[23]. There are some studies addressing the regulation of pituitary hormones by orexinergic system[24,25], but the mechanism how other hormonal milieu affects the orexinergic system, is not yet clear. Silveyra et al had determined the expressions of hypothalamic and pituitary levels of PPO, OX1R and OX2R in female Sprague-Dawley rats at various estrous stages correlating the same with the endocrine milieu, food intake and light-darkness cycle. The report suggests that expression of OX1R and OX2R increases in both the hypothalamus and pituitary, during proestrus phase while the expressions remain unaltered in estrus or diestrus. Moreover, the hypothalamic PPO expression had shown increase only during the proestrus phase[26]. Nitkiewicz *et al*[27] have also compared the expression of the *PPO* gene in porcine endometrium and myometrium and the intensity of OXA-and OXB-immunoreactivity in endometrial glandular and luminal epithelium and stroma as well as the myometrial longitudinal and circular muscles during the four stages of the estrous cycle. The highest *PPO* mRNA expression was observed in the endometrium and myometrium on days 14-16 of the estrous cycle. The myometrial *PPO* gene expression was vivid than in the endometrium on days 2–3 of the cycle, while the endometrial gene expression was markedly higher in later phase (days 17–19)[27].

Expressions of *PPO* mRNA and OX1R, but not OX2R were found in different cells in the rat testis[19,28]. Whereas, in human testis, *OX1R* and *OX2R* mRNA expressions but not *PPO* mRNA were found[13]. *In vitro* slice preparation and *in vivo* experiments demonstrated that OXA could stimulate basal testosterone secretion[18,28–30]. These studies also provided hints regarding low *OX1R* gene expressions also in the Leydig cells[13,20,29,31]. Studies revealed that orexins operate through activation of the phospholipase Cpathway *via* induction of inositol triphosphate production, and in this regard, OXB have been shown to be more potent.

4. Role of orexins in modulation of HPG axis

The HPG axis is the principal endocrine axis for the regulation of reproductive functions, and is under the influence of several other hormonal and neuronal crosstalks such as thyroid[32-34], kisspeptin[35], melatonin[36], other metabolic hormones[37], *etc*. Orexin immunoreactive fibers have overlapping distributions with the GnRH neuronal system in the septo-preoptic and the arcuate nucleus-median eminence region that is suggestive of the influence of orexins in the modulation of pituitary luteinizing hormone (LH) secretion *via* regulation of GnRH release[38]. Although the studies on the effect of orexins upon the HPG axes mostly used female rodent as laboratory model, the overall observation suggests that orexins greatly influence GnRH neurons and their secretions to regulate reproductive functions *via* alterations in the HPG axis[38]. Since orexins alter the pulse of GnRH release, they play a vital role in regulating LH secretion from the anterior pituitary that in turn may modulate steroidogenesis in the Leydig cells in male, thereby affecting testicular functions.

Pu *et al*[24] demonstrated that OXA or OXB injection through intracerebroventricular route could stimulate LH secretion in a dose- and time-related fashion in estradiol benzoate and progesterone pre-treated ovariectomized rats. However, the effects of exogenous orexins injection were shown to depend upon the status of ovarian steroids. It has been observed that ovariectomized rats treated with 17β-estradiol and progesterone followed by orexins injection, showed an increased plasma LH levels which may suggest that estrogen upregulates orexin receptors[39]. Orexin-mediated LH response in the hypothalamus appears to be site-specific and be carried out by OX1R, located on the GnRH cells[38]. Thus, orexins may potentially stimulate GnRH from the hypothalamus, but is dependent upon other steroidal interference.

In sheep, orexinergic neurons are mostly found in the dorsomedial hypothalamic nucleus, zona incerta, lateral hypothalamic and perifornical areas[40]. A substantial number of GnRH cells are in close contact with the orexin immunoreactive terminals which indicate a role of orexins in the regulation of GnRH cells. Hypothalamic areas concerned with neuroendocrine functions also have been reported to express orexin receptor mRNAs[40].

Orexins can indirectly act through β-endorphins (endogenous opioid peptides) to suppress GnRH secretion[41]. Orexin neurons course through the arcuate nucleus innervating the proopiomelanocortin neurons, which are β-endorphin precursors. It had been shown that co-administration of naloxone, an opioid antagonist, with orexins reversed the effect of orexins on the mean LH concentration and the GnRH pulse frequency. This observation suggests that β-endorphin is involved in actions of orexins to reduce LH concentration and hypothalamic GnRH release pulse frequency[41]. Unlike OXA, naloxone had no influence upon OXB which still significantly suppressed LH level and its pulse frequency. Hence, OXB perhaps is not dependent upon β-endorphin pathway for its effects on hypothalamic GnRH secretion. Since orexins have been seen to regulate the anterior pituitary responsiveness towards GnRH for LH secretion, it may be suggested that gonadotrophs express a considerable number of orexin receptors. The somatotrophs and corticotrophs already have been proven to bear both the orexin receptors[41].

5. Orexins, obesity and testicular functions

The word 'orexin' is adapted from the Greek word referring to 'appetite'. Activation of the orexin system can increase both spontaneous physical activity and food intake, and its primary function is to promote an increase in energy expenditure[42,43]. While obesity and overweight have turned pandemic over recent years, there are individuals who may naturally resist obesity. Reports suggest that orexins or hypocretins may be a key brain component that mediates the mechanism of obesity resistance[44,45]. Research on obesity resistance in animal models has demonstrated positive correlations of increased orexins in spontaneous physical activity. This orexin-induced spontaneous physical activity has been postulated to be a major contributor to obesity resistance through enhanced non-exercise activity thermogenesis[46]. However, the underlying mechanism of how central hypothalamic orexin signaling regulates spontaneous physical activity is not yet completely revealed.

Obesity presents have several physiological disturbances, of which impairment in normal reproductive functions being one such issue of major concern[47,48]. It is suggested that obesity positively associates with male infertility[49,50]. In an attempt to clarify the mechanism by which obesity affects male fecundity and semen quality, the role of various obesity-related hormones in regulation of male reproductive functions have been studied, such as adiponectin, obestatin, ghrelin, leptin among others[37].

It has been discussed in previous sections that orexins have been reported to greatly influence GnRH neurons and their secretions. Several studies using rodent models indicate that orexins increase significantly the aromatase (*Cyp19*) gene expression in the hypothalamus of male rats. Aromatase is an enzyme which converts androgens to estradiol in the hypothalamus. Aromatase cytochrome P450 is an enzyme coded by *Cyp19* gene. This enzyme converts androgens like testosterone to estradiol in peripheral tissues and in the brain[41,51]. It is further noted that hypothalamic interneurons-including neuropeptide Y, pre-opiomelanocortin or ghrelin may play a role in mediating the inhibitory effects of orexins on HPG axis[21, 52,53], which is a complex integrated network influenced by central and peripheral signals.

Previous research has shown that the central injection of orexins significantly increased the *Cyp19* gene expression and estradiol hormone levels in the hypothalamus of male rats[54]. Orexins are hypothalamic neuropeptides which mainly poise inhibitory effects on reproductive axis[24,55]. OXA has been shown to decrease the mean serum level of the LH and testosterone[56]. Orexins may regulate the reproductive axis by influencing secretions of GnRH and LH[11]. From the umpteen animal research it is evident that peptides like orexins are important in the regulation of testicular functions[57].

Recent data have thrown lights upon a significant role of orexins,

especially OXA, in the regulation of male reproductive functions owing to their receptor expressions in vital testicular cells, such as the Leydig cells, Sertoli cells as well as spermatozoa at different developmental stages, even in the epididymis and penis[13,58,59]. It has also been reported that increase in orexins dosage to rats highly stimulated testosterone secretion from testis[29]. Adrenal glands as well as the gonads, require four key steroidogenic enzymes to synthesize testosterone, among which the 3β-hydroxysteroid dehydrogenase (3β-HSD) is considered the most important[60]. The 3β-HSD expression has been detected in several organs[61-63] and is also an immunohistochemical marker to determine testosterone synthesis. Orexins have been shown to stimulate 3βHSD expression in adrenocortical cells[64] and in rat primary Leydig cells[65] suggesting that orexins may regulate steroidogenesis in steroidogenic cells. The expression of functional orexin receptors in testicular peritubular myoid cells and the induction of phospholipase C/inositol triphosphate cascade may promote further testicular functions. Orexins thus possess differential impacts upon each male reproductive tissue, and the pleiotropic effects of orexin receptors may be suggested to involve multiple signaling pathways. It has been hypothesized that orexins may influence testosterone production *via* the orexin receptors in Leydig cells, which again may regulate expression of orexin receptors in other tissues, thereby establishing a positive feedback loop[13].

6. Orexins and male reproductive behavior

The cell bodies of neurons with HCRTs/OXs are present in the lateral and dorsal hypothalamus and project to various areas of the brain concerned with sexual behavior. These areas include the medial preoptic area, paraventricular nucleus, and the ventral tegmental area[9]. In a pioneer study showcasing the possible functions of orexins in sexual behavior showed that HCRT1/OXA administration into the medial preoptic area enhanced sexual arousal and copulatory performance[66]. Consequent studies revealed an increase in immunoreactivity in hypocretin/orexin neurons during copulation in male rats[67]. Moreover, systemic administration of an OX-1 receptor antagonist in rats was shown to downregulate copulatory behavior. It had been suggested that orexins may also act in a steroid-sensitive fashion to aid the rewarding sensation of natural stimuli such as sex, by activating the mesolimbic dopaminergic system[67]. This concept has been challenged by specific studies on the role of orexin neurons in sexual behavior in male rats using particular neural activation markers and selective neuronal lesions[68]. The net findings of these studies are that activation of orexin neurons increases when a receptive or non-receptive female is presented without any further activation, or when there are cues predicting sexual reward. While, orexin neuron lesions decreased the latencies to mount and intromission during the first mating trials. It can thereby be put forth that orexins may not be vital for male sexual performance but is essential in sexual arousal mainly in naive animals and may be critical for sexual reward processing[68].

The above studies implicate the participation of orexins in steroidogenesis, spermatogenesis, transportation and maturation of sperm and in the control of penile function including the maintenance of penile erection. A substantial number of researches are required in this direction to elucidate the mechanisms of orexins action on different testicular functions[13] and most importantly on the effect of orexins on semen quality, which is the universally accepted parameter to gauge male fertility[69-73].

7. Conclusions

Orexins, discovered not long ago, are the molecules of high interest in research due to their versatility. Besides, its crucial role in the regulation of energy balances, reward systems, emotions, and arousal, emerging data suggest direct effects of orexins upon the GnRH neurons and regulation of reproduction. Most of the studies on orexins demonstrated the anatomic architecture of the orexinergic system and its role in some key peripheral functions, either by the actions of orexins on central control system or directly *via* its interaction with the peripheral effectors. Despite substantial evidence on the expression of orexin receptors in hypothalamus, anterior pituitary and testicular cells like the Leydig cells, Sertoli cells and spermatozoa at different developmental stages, the exact physiological role of orexin in male reproduction needs a lot more investigations. There are several open questions regarding the roles of orexins on HPG axis, modulation of male reproductive hormones and their signal transduction pathways. Nevertheless, it may be hypothesized that orexin may act upon the hypothalamus and pituitary to modulate the influence of HPG endocrine axis upon testicular functions; or it may act directly *via* its receptors in major testicular cells like Leydig cells and Sertoli cells to regulate steroidogenesis and spermatogenesis; and even may act on the germ cells to determine their viability and development. Altogether, the existing evidences suggest that the orexinergic system might act as a common association among crucial functions like reproduction with other centrally controlled functions like energy balance, alertness and the inner biological clock.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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