

## Orexin A and its role in the regulation of the hypothalamo-pituitary axes in the rat

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### SUMMARY

Orexin A (OxA), a recently discovered neuropeptide, is synthesized mainly by neurons located in the posterolateral hypothalamus and is a 33 amino acid peptide with N-terminal pyroglutamyl residue and two inter-chain disulfide bonds. It is a potent agonist for both the orexin-1 (OxR1) and orexin-2 (OxR2) receptors. Orexin A and its receptors are widely distributed in the central nervous system (CNS) and peripheral organs suggesting the pleiotropic functions of this peptide. Orexin A is involved in food intake and energy expenditure in many species, but also plays an important role in the regulation of the hypothalamo-pituitary axes. The role of orexin A in the regulation of the hypothalamo-pituitary-adrenal, -thyroid, -somatotrophic, and -gonadal axes has been inadequately investigated. Orexinergic

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fibres project to the septal-preoptic and arcuate nucleus-median eminence regions - two areas of the brain directly involved in the synthesis and release of gonadotropin-releasing hormone (GnRH). Contentious opinions concerning the influence of orexin A over the hypothalamo-gonadotropic axis have been reported in both *in vivo* and *in vitro* studies. Further studies are necessary to clarify relationships between orexin A and the hypothalamo-pituitary hormones involved in reproduction. *Reproductive Biology* 2006 6 Suppl. 2:29–35.

**Key words:** orexin A, hypothalamo-pituitary axes, GnRH/LH

### **Orexin A – properties and the localization**

Orexin A (OxA), a recently discovered peptide, is synthesized in the central nervous system (CNS) mainly by neurons located in the posterolateral hypothalamus [2,17]. In the rat, both orexins A and B are derived from 130 amino acid precursor prepro-orexin. OxA is a 33 amino acid peptide with N-terminal pyroglutamyl residue and two inter-chain disulphide bonds. The sequence of OxA is conservative and identical in many species like human, mouse, rat, bovine and porcine [22]. Furthermore, it has been reported that OxA is able to cross the blood-brain barrier [8]. The orexinergic neurons are widely distributed in the CNS, mainly in the cortex, hippocampus, septum, thalamus, hypothalamus, cerebellum, brain stem and spinal cord [12, 13]. They have been also found in a variety of peripheral organs and endocrine glands including the gastrointestinal tract, pancreas, adrenal, testis, pineal and pituitary glands, and sympathetic neurons [10].

### **Orexin A receptors**

The action of OxA is mediated by two different receptors, OxR1 and OxR2, both coupled with protein-G. The OxR2 binds both orexins A and B with similar potency, while OxR1 is selective for OxA [10, 17]. The receptors are widely distributed in the CNS. The OxR1 is located in the ventromedial and lateral hypothalamus, hippocampus, locus coeruleus, pineal and pituitary gland, whereas OxR2 is present in the thalamus, hypothalamus,

septum, cortex and brain stem. The receptors for OxA are also expressed in peripheral tissues e.g. the gut, pancreas, adrenal gland, thyroid, kidney, testis and lung [10]. The wide distribution of OxA and its receptors in the CNS and peripheral organs may suggest the pleiotropic functions of this peptide.

### **Functions of orexin A**

The first identified functions of OxA have been connected to its participation in the feeding behaviour and sleep/awake activity [4, 10]. The orexinergic neurons are found mainly in the lateral hypothalamus and locus coeruleus, where respectively feeding and wake centres are located [4, 22, 23]. OxA could be considered not only as a neurotransmitter and/or neuromodulator, but also as a hormone due to the secretion of this peptide into the circulating blood [10]. It has been found that OxA influences the hypothalamic and pituitary hormone release in rats. It has been shown that intracerebroventricular (icv) administration of OxA inhibits prolactin release and this effect is partially independent of the dopaminergic system [15]. Moreover, the prolactin secretion is reduced during fasting through up-regulated activity of the central OxA system [5].

It has been published that OxA also exerts an effect on the corticotropin-releasing hormone (CRH) – adrenocorticotropin (ACTH) axis. Centrally administered OxA enhances ACTH release in a dose-dependent manner through an increase of CRH secretion [7, 19]. On the contrary, *in vitro* studies revealed the inhibitory effect of OxA on CRH-stimulated ACTH secretion [18].

It has also been reported that OxA modulates growth hormone (GH) release. Intracerebroventricular administration of OxA decreases basal GH secretion in the rat but does not change the GH response to the growth hormone – releasing hormone (GHRH; [11]). However, GH secretion, both in basal conditions and in response to GHRH, is unmodified in *in vitro* studies [20].

The influence of OxA on the secretion of thyrotropin – releasing hormone (TRH) and thyroid stimulating hormone (TSH) has been noted. Intravenous

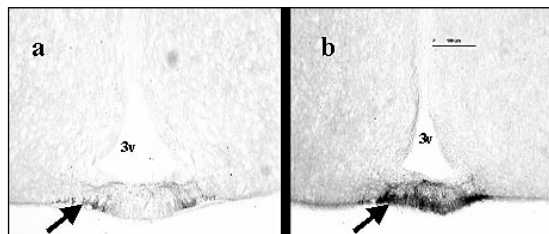
injections of OxA trigger an increase of the hypothalamic TRH content and decrease the level of plasma TSH. The plasma thyroid hormone's concentrations showed no changes. Intracerebroventricular infusions of OxA also reveal the inhibitory effect on TSH secretion, while the plasma levels of thyroid hormones remain unchanged [10].

### **Orexin A and reproduction**

It has been found that immunoreactive (ir) orexinergic fibres project from the lateral hypothalamus to the septal-preoptic and arcuate nucleus-median eminence regions [13]. These areas are directly involved in the control of the hypothalamo-gonadotropic axis through the synthesis and release of the gonadotropin-releasing hormone (GnRH). Thus, orexinergic neurons may potentially play an important role in the regulation of the hypothalamo-gonadotropic axis. Contentious opinions have been reported concerning the changes in GnRH/LH secretion after OxA treatment, both in *in vivo* and *in vitro* studies. OxA has an inhibitory effect on luteinizing hormone (LH) secretion by influencing GnRH release [16]. Additionally, it has been previously shown that OxA significantly reduces the mean concentration of serum LH and the pulse frequency in ovariectomized (OVX) rats [6]. Furthermore, OxA has revealed the bimodal effect; it either stimulates LH secretion in steroid-primed OVX rats, or suppresses LH secretion in non-primed OVX subjects [1, 3, 21]. On the other hand, the high hypothalamic concentration of OxA may contribute to the LH surge during the proestrous phase [14]. Additionally, Kohsaka et al. [9] have observed that icv administration of OxA to fasted OVX rats resulted in a dose-dependent preovulatory LH surge.

Based on *in vitro* experiments it has been shown that OxA could bring on a release of GnRH from hypothalamic explants taken from rats during proestrous but not estrous or metestrous phases [14]. On the contrary, results from an *in vivo* study suggest that OxA may suppress GnRH secretion probably *via* the  $\beta$ -endorphin system [6]. It has been found that approximately 80% of GnRH neurons were contacted with orexinergic fibres. Approximately 85% of GnRH neurons are also co-localized with

both orexin receptors [14]. These results suggest that OxA can modulate GnRH neurons activity directly via O<sub>x</sub>R<sub>s</sub>. An interaction between OxA and neuropeptide Y (NPY) can be connected with receptor Y<sub>1</sub> of NPY. It has been shown by Russell and co-workers [16] that the specific Y<sub>1</sub>NPY receptor antagonist abolishes OxA stimulated GnRH release *in vitro* [1, 16]. The orexin neurons also show a co-expression with the Y<sub>4</sub>NPY receptor but these neurons do not make close contacts with GnRH neurons. These results suggest that OxA can indirectly modulate GnRH neurons by stimulation of the Y<sub>4</sub>NPY receptor which is involved in LH release [14]. According to our knowledge, there is a lack of data describing the influence of OxA on GnRH release in immature animals. Our preliminary, unpublished results suggest that the observed increase of immunoreactive GnRH (irGnRH) in the median eminence after icv OxA infusion may be due to restraining the neurosecret in the nerve terminals (fig. 1). Thus, OxA can suppress the release of GnRH, and in consequence, may decrease gonadotropins secretion. The preliminary results suggest that OxA reduces the activity of the hypothalamo-gonadotropic axis in immature female rats. Summarizing, it should be emphasized that OxA shows a wide distribution in the CNS, and possesses some neuromodulatory and/or hormonal functions. Cumulative data indicate that OxA can play an integrative role in the control of metabolic, nutritional and reproductive processes. Further studies are necessary to clarify all relationships between OxA and the hypothalamo-pituitary axes.



*Figure 1.* Representative image of the immunoreactivity of the gonadotropin-releasing hormone (black arrow) in the medial part of the median eminence observed 1 hour after intracerebroventricular infusion of artificial cerebrospinal fluid [a] or 1  $\mu$ g of OxA [b] to immature, 30 days old female rats ( $n=5$  for each group). 3v – the third ventricle of the brain. Scale bar: 100  $\mu$ m

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