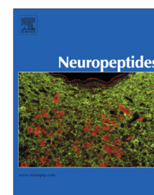
Neuropeptides 48 (2014) 199–205

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/npep



# Effect of orexin A on the release of GnRH-stimulated gonadotrophins from cultured pituitary cells of immature and mature female rats

L. Martynska [a], E. Wolinska-Witort [a], M. Chmielowska [a], M. Kalisz [a], B. Baranowska [b], W. Bik [a,*]

[a] Department of Clinical Neuroendocrinology, The Centre of Postgraduate Medical Education, Warsaw, Poland
[b] Department of Clinical Physiology, The Centre of Postgraduate Medical Education, Warsaw, Poland

ARTICLE INFO

*Article history:*
Received 2 December 2013
Accepted 20 May 2014
Available online 28 May 2014

*Keywords:*
Orexin A
Gonadotrophins
GnRH-stimulated pituitary cells
Female rats

ABSTRACT

Orexin A (OxA), also known as hypocretin 1, is a regulatory neuropeptide involved in the control of various autonomic and neuroendocrine functions. It appears to have a significant impact on the regulation of trophic hormones secretion by influencing the hypothalamus and the pituitary. Orexin A acts through two types of receptor found in the pituitary. This suggests the possibility of direct action of OxA at the adenohypophysis level.

The aim of this study was to investigate the direct effect of OxA on GnRH (gonadotrophin-releasing hormone)-stimulated LH and FSH secretion from cultured pituitary cells of sexually immature and mature female rats.

Anterior pituitary cells obtained from immature and mature female rats (ovariectomized, and ovariectomized and treated with estradiol) were incubated with $10^{-10}$ M or $10^{-7}$ M orexin A for 1hour and 4 h and the effect on GnRH-stimulated ($10^{-9}$ M or $10^{-6}$ M) LH and FSH release was examined. The concentrations of secreted gonadotrophins in the culture media were determined by RIA methods.

Orexin A significantly inhibited GnRH-stimulated FSH release from pituitary cells isolated from immature female rats, whereas in cells of mature ovariectomized animals, the effect of OxA was dependent on the stimulatory dose of GnRH. When the cells were stimulated with a low dose of GnRH, orexin A inhibited the secretion of gonadotrophins, but when a high dose of GnRH was used, orexin A increased mainly the release of LH. In cultured pituitary cells from ovariectomized, estrogenized mature rats, orexin A inhibited the secretion of LH if the cells were stimulated with a high dose of GnRH.

In conclusion, the results of this study revealed that orexin A may modify the sensitivity of gonadotrophic cells to GnRH, and its effect depends on the maturity and estrogen status of the rats from which the cells are isolated.

© 2014 Elsevier Ltd. All rights reserved.

## 1. Introduction

Orexin A (OxA), also known as hypocretin 1, is a peptide synthesized mainly by neurons with perikarya located within and around the lateral and posterior hypothalamus (de Lecea et al., 1998; Sakurai et al., 1998). These neurons form an extensive system in the CNS (Kukkonen et al., 2002; Kukkonen, 2013). Orexin A acts through two different G-protein coupled receptors, OX1R and OX2R. This neuropeptide is a selective endogenous agonist of OX1R, whereas OX2R binds both orexin A and another peptide, orexin B (Sakurai et al., 1998). Orexin receptors are present in various areas of the brain and peripheral tissues, e.g. pituitary gland and ovary (Date et al., 2000; Voisin et al., 2003; Sakurai, 2005; Silveyra et al., 2007a, 2007b). The wide occurrence of OxA and its receptors indicates that this peptide may have pleiotropic effects. Orexin A is involved in the regulation of sleep/wakefulness, energy homeostasis and locomotor activity (de Lecea et al., 2002; Sakurai et al., 1998; Rodgers et al., 2002; Nishino, 2007; Ohno and Sakurai, 2008; Sakurai, 2007; Chung and Civelli, 2006; Hoyer and Jacobson, 2013). It also appears to have a significant impact on the regulation of tropic hormones secretion on the level of the hypothalamus and pituitary (López et al., 2010). It has been demonstrated that orexinergic fibres have connections with about 80% of GnRH neurons, and that approximately 85% of GnRH neurons express both types of orexin receptor (Campbell et al., 2003). These findings indicate that OxA may have a direct influence on these neurons. On the other hand, orexin expression was detected in the pituitary (Date et al., 2000). Moreover, the presence of orexin receptors has also

* Corresponding author. Address: Department of Clinical Neuroendocrinology, The Centre of Postgraduate Medical Education, Marymoncka 99/103, 01-813 Warsaw, Poland. Tel.: +48 22 56 93 850; fax: +48 22 56 93 859.
*E-mail address:* wojtmed@wp.pl (W. Bik).

http://dx.doi.org/10.1016/j.npep.2014.05.005
0143-4179/© 2014 Elsevier Ltd. All rights reserved.

been demonstrated in the anterior pituitary (Blanco et al., 2001; Jöhren et al., 2003; Silveyra et al., 2007b). These data imply that orexin can act on the pituitary gland as a hormone, in addition to the modulatory function of OxA at the hypothalamus level.

Several studies have shown that OxA plays a regulatory role in the reproductive axis. Intracerebroventricular administration of orexin was found to stimulate LH secretion in steroid-primed ovariectomized (OVX) rats (Pu et al., 1998), but suppressed LH secretion in unprimed OVX rats (Tamura et al., 1999). It has been reported that the activity of the orexin system in the hypothalamus, pituitary and ovaries is associated with the hormonal milieu of the estrous cycle (Silveyra et al., 2007a, 2007b; Kaminski et al., 2010; Nitkiewicz et al., 2010). It is also known that the sexual maturation process is accompanied by increased pulsatile secretion of GnRH. GnRH secreted from nerve endings in the median eminence of the hypothalamus is the main stimulator of the transcriptional activity of genes encoding gonadotrophin subunits and the release of these hormones from the pituitary (Burger et al., 2004). Sexual maturity is associated with an increased frequency of pulsatile GnRH secretion. However, the mechanism controlling the secretion of this hormone during puberty is complex and remains unclarified (Terasawa and Fernandez, 2001; Ojeda et al., 2006).

Orexin A may also modulate the activity of GnRH neurons and gonadotrophin-secreting pituitary cells (Silveyra et al., 2010). GnRH neuronal activity and the synthesis and secretion of LH and FSH from the pituitary depends not only on the effects of neuropeptides secreted in the CNS, but may also be associated with the degree of sexual maturity and the hormonal status (Ciechanowska et al., 2010; Terasawa and Fernandez, 2001).

There is some controversy regarding the influence of orexin A on the hypothalamo-gonadotrophic axis, both *in vivo* and *in vitro*. Further studies are required to clarify the relationship between orexin A and the hypothalamo–pituitary hormones involved in reproduction.

The aim of this *in vitro* study was to determine whether the administration of orexin A alters the response of gonadotrophs of immature and mature female rats to gonadotrophin-releasing hormone.

## 2. Material and methods

### 2.1. Animals

This study was conducted on female Wistar rats obtained from Laboratory Animal Breeding in Warsaw. Animals were kept for 7 days under controlled temperature (23 ± 1 °C) and lighting (LD 14:10) conditions, with the light turned on at 06:00. They were housed in standard cages with free access to water and standard pelleted food (Murigran, Poland). All experiments were approved by the 1st Warsaw Ethics Committee for Experiments on Animals.

The following experimental rat models were used: sexually immature females (IM, aged 25 days, body mass 90–110 g, $n = 30$) and sexually mature females (M, aged 3 months, body mass 220–260 g, $n = 60$) that were either ovariectomized and treated 7 days post-surgery with 200 μl of oil administered subcutaneously (sc) for 3 consecutive days (M/OVX) or ovariectomized and given 17-β estradiol supplementation (20 μg/200 μl oil sc/rat) for 3 consecutive days preceding the excision of pituitary gland (M/OVX + $E_2$). Removal of the gonads (ovariectomy) performed on the two latter experimental groups is a procedure that eliminates endogenous estrogens of ovarian origin. Subcutaneous injections of oil (M/OVX) or estradiol (M/OVX + $E_2$) in mature females after ovariectomy permitted standardization of the estrogen level within these experimental groups. On the day of the pituitary collection the rats were anesthetized by intraperitoneal injection of ketamine and then decapitated. The pituitary glands were quickly dissected under sterile conditions.

### 2.2. Cell culture technique and experimental protocol

Anterior pituitary cells isolated from each group of the experimental rats (IM, M/OVX, M/OVX + $E_2$) were cultured with or without OxA to determine whether their secretion of LH and FSH was altered in response to a GnRH stimulus. The procedures used for pituitary tissue dissociation, cell preparation and cell culture were those previously described in details by Martynska et al. (2011). Briefly, the anterior pituitaries were enzymatically and mechanically dispersed and cultured. The pituitary cells were counted in a hemocytometer and assessed for viability by trypan blue exclusion (>97%). The isolated cells were diluted to $5 \times 10^5$ cells/ml with DMEM culture medium and plated at 1 ml cell suspension/culture plate well. The pituitary cells were incubated at 37 °C in a humidified atmosphere containing 5% $CO_2$ and the growth medium was changed 48 h after seeding. On the 3rd day of culture, the cells were covered with 1 ml of fresh serum-free DMEM medium. Experiments were then conducted to determine the basal levels of LH and FSH secreted by untreated anterior pituitary cells in culture (control) as well as the effect of $10^{-10}$ or $10^{-7}$ M orexin A on GnRH-stimulated ($10^{-9}$ or $10^{-6}$ M) gonadotrophin secretion. The required additions were made to the growth medium for the separate treatments and all cells were then incubated for 1 hour and 4 h before collection of the media and storage at −20 °C prior to analysis.

### 2.3. Radioimmunoassays

The concentrations of LH and FSH in growth media were determined by radioimmunoassays performed using reagents prepared by Dr. A.F. Parlow and provided by the NIDDK (Bethesda, MD, USA). Results were expressed relative to LH-RP-3 and FSH-RP-2 reference standards. The cross-reactivity of rLH antiserum with other anterior pituitary hormones was negligible (<0.01%). The FSH antibody cross-reacted slightly with rTSH (1.7%), rPRL (0.06%), rGH (0.02%) but did not cross-react with rLH (<0.01%). The assay sensitivities were 0.1 ng/ml for LH and 1.25 ng/ml for FSH. All measurements were made twice in one assay for each hormone. Intra-assay coefficients of variation (CV) were less than 7%.

### 2.4. Materials

All media and chemicals used for cell culture as well as OxA (Cat. No. O6012) and GnRH (Cat. No. L8008) were purchased from Sigma (Sigma–Aldrich, Germany). 24-well culture plates were obtained from Nunc (Thermo Fisher Scientific, Denmark).

### 2.5. Statistical analyses

All data are expressed as the median and interquartile range +/− min/max. Statistical analyses were performed using Statsoft Statistica 7.1 PL for Windows. Initially, all groups of data were tested for normality using the Shapiro–Wilk's test. Statistical differences between the groups were determined by the non-parametric Kruskall–Wallis test followed by the Mann–Whitney *U* test. Differences between mean values were considered significant when $p < 0.05$.

## 3. Results

### 3.1. Effect of orexin A on $10^{-9}$ M GnRH-stimulated LH release from cultured anterior pituitary cells

The administration of $10^{-9}$ M GnRH to primary cultures of IM female pituitary cells increased the level of LH released into the

Download English Version:

https://daneshyari.com/en/article/2808055

Download Persian Version:

https://daneshyari.com/article/2808055

Daneshyari.com