Acta Histochemica 116 (2014) 286–288

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Short communication

Orexin 1 receptor in the seminiferous tubules of boar testis: An immunohistochemical study

Finizia Russo[a], Lucianna Maruccio[b,*], Antonio Calamo[b], Paolo de Girolamo[b], Ettore Varricchio[a]

[a] Department of Biological, Geological and Environmental Sciences, University of Sannio, Via Port'Arsa 11, 82100 Benevento, Italy
[b] Department of Veterinary Medicine and Animal Productions, University of "Federico II", Via Veterinaria 1, 80137 Napoli, Italy

ARTICLE INFO

Article history:
Received 9 January 2013
Received in revised form 23 April 2013
Accepted 25 April 2013

Keywords:
Orexin receptor 1
Male genital tract
Immunohistochemistry
Pig

ABSTRACT

Orexin receptor 1 (OX_1R) and orexin receptor 2 (OX_2R) are two G-protein-coupled receptors that bind their ligands, orexin A (OXA) and B (OXB), with different affinities. The male genital system represents an important target for OXA, which appears to play a role in the control of steroidogenesis and germ cell development in the testis. It is known that among domestic breeding animals, in the boar the number of Leydig cells is very high and OXA appears to have stimulatory activity on testosterone production. In this study, we aimed to evaluate the presence of OX_1R in the boar testis in order to extend our knowledge concerning the distribution and a potential functional role of the orexinergic system in the male reproductive tract of farm animals. The presence of OX_1R immunopositive cells in seminiferous tubules of the boar testis enables us to hypothesize a possible role of OXA on male germ cells cycle in pig. Further investigations, involving functional and ultrastructural analysis, may contribute to our understanding of the role of orexins in the boar genital system.

Orexin A (OXA) and orexin B (OXB) were initially identified as hypothalamic neuropeptides derived from the same precursor prepro-orexin (de Lecea et al., 1998; Sakurai et al., 1998). The biological actions of these peptides are mediated through two closely related G-protein-coupled receptors: orexin receptor 1 (OX_1R) and orexin receptor 2 (OX_2R). However, while OX_1R is highly selective for OXA, OX_2R binds both orexins with similar affinity (Sakurai et al., 1998). Orexigenic fibers are widely distributed and project to multiple brain regions (Peyron et al., 2000), thus suggesting the involvement of orexins in the central control of various biological functions such as the sleep–wake cycle (Piper et al., 2000), sexual behavior and arousal (Gulia et al., 2002) and several neuroendocrine axes, including corticotrope, lactotrope, somatotrope, and gonadotrope systems (Kuru et al., 2000; Kohsaka et al., 2001; Russell et al., 2001; Overeem et al., 2003).

Several studies have demonstrated that both orexins and their receptors are also expressed in peripheral organs belonging to the gastrointestinal tract (Kirchgessner and Liu, 1999; Ehström et al., 2005; Dall'Aglio et al., 2009, 2012) and the urogenital tract (Karteris et al., 2004; Barreiro et al., 2005; Takahashi et al., 2005; Russo et al., 2008). In particular, the expression of OXA, prepro-orexin and the cognate receptors (OX_1R and/or OX_2R) in the human, rat, sheep, South American camelid Alpaca and fowl male gonads has been reported (Johren et al., 2001; Ohkubo et al., 2003; Karteris et al., 2004; Barreiro et al., 2005; Zhang et al., 2005; Liguori et al., 2013). OXA also appears to be involved in the control of steroidogenesis and germ cell development in the interstitial compartments of the testis (Barreiro et al., 2004, 2005; Nurmio et al., 2010). Among breeding domestic animals, it is known that in boar the number of Leydig cells is very high. In this study, we aimed to evaluate the presence of OX_1R in the boar testis in order to extend our knowledge concerning the distribution and a potential functional role of the orexinergic system in the male reproductive system of farm animals.

Samples of testis were collected from five healthy breeding boars, immediately after their castration in local farms (Campania region, Italy). 1 cm^3 cubes of tissue were fixed in Bouin's fluid for 24 h, dehydrated through ascending ethanol series and embedded in Paraplast Plus (Leica Biosystems, Richmond, IL, USA). 7 μm-thick sections were cut by microtome, collected on slides, and stained by the avidin–biotin immunohistochemical technique. In the specific step, a polyclonal antibody raised against OX_1R (sc-8073, Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used, diluted 1:200 and applied on sections overnight at 4 °C. The other components of the immunological reaction were contained in the Vectastain Elite ABC Kit (PK-6105, Vector Laboratories, Burlingame, CA, USA). The final staining was performed using a solution of 3,3′ diaminobenzidine tetrahydrocloride (Sigma–Aldrich, St. Louis, MO, USA) of 10 mg in 15 mL 0.5 M Tris buffer, pH 7.6, containing 0.03% hydrogen peroxide. An antigen unmasking procedure preceded

* Corresponding author.
E-mail address: lucianna.maruccio@unina.it (L. Maruccio).

0065-1281/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.acthis.2013.04.012

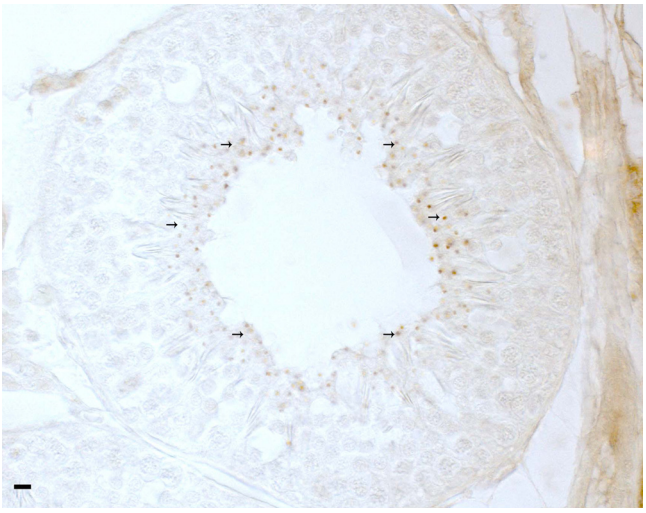

Fig. 1. Digital reconstruction of 13 micrographs in sequence of one boar seminiferous tubule. OX_1R immunopositive oval spermatids (arrows). The immunoreactive granules were localized in the peripheral of acrosomal bodies and were punctiform in shape. Bar = 10 μm.

the immunohistochemical reaction and was carried out by dipping the sections in 0.01 M sodium citrate buffer, pH 6.0, and heating them in a microwave oven for 10 min at 750 W. Negative controls were obtained by substituting the primary antisera with PBS or normal serum in the specific step, or alternatively, by absorbing each primary antiserum with an excess of the relative peptide (100 mg of peptide/ml of diluted antiserum). The immunostained section were photographed using a Leica DMRA2 microscope (Leica Microsystems, Wetzlar, Germany) equipped with a DC300F digital camera.

OX_1R immunopositive cells were detected in the seminiferous tubules of the boar testis. In particular, numerous acrosomal bodies of round, oval (Fig. 1, arrows) and elongated (Fig. 2A, arrows) spermatids were immunostained. In round spermatids OX_1R immunopositive granules were semilunar in shape and located perinuclearly, while in oval and elongated ones, they appear punctiform in shape and located peripherally. In each positive seminiferous tubule, OX_1R immunoreactive spermatids were observed at the same stage of the developmental cycle (Figs. 1 and 2A). No immunopositivity was found in interstitial compartments (Fig. 2A, asterisk). In negative control sections no OX_1R immunoreactivity was detected (Fig. 2B).

In agreement with previous reports (Barreiro et al., 2004; Assisi et al., 2012), immunohistochemical staining of boar testis revealed OX_1R-containing cells in the tubular compartment of the gonad. In the rat testis, OX_1R mRNA levels varied significantly depending on the developmental stage, with peak values in the testes from neonatal and pubertal to early adult (Barreiro et al., 2004). Also, in line with the notion of the predominantly tubular location of the transcript, Leydig cells elimination by cytotoxic treatment *in vivo* did not have any significant effect on the OX_1R mRNA levels for the first 15 days after cytotoxic administration, which suggests that Leydig cells are not the major source of OX_1R expression (Barreiro et al., 2004). In the rat testis no expression of OX_2R mRNA has been detected (Barreiro et al., 2004); whereas human and sheep testes have been demonstrated to express OX_1R and OX_2R (Karteris et al., 2004; Zhang et al., 2005). Similarly, expression of orexin receptors has also been reported in the chicken testis (Ohkubo et al., 2003). In humans, testicular immunostaining of OX_1R has been detected in Leydig cells, peritubular myoid cells and in Sertoli cells (Karteris et al., 2004). The available data strongly suggest that male gonad is a potential site for expression and/or biological actions of orexins.

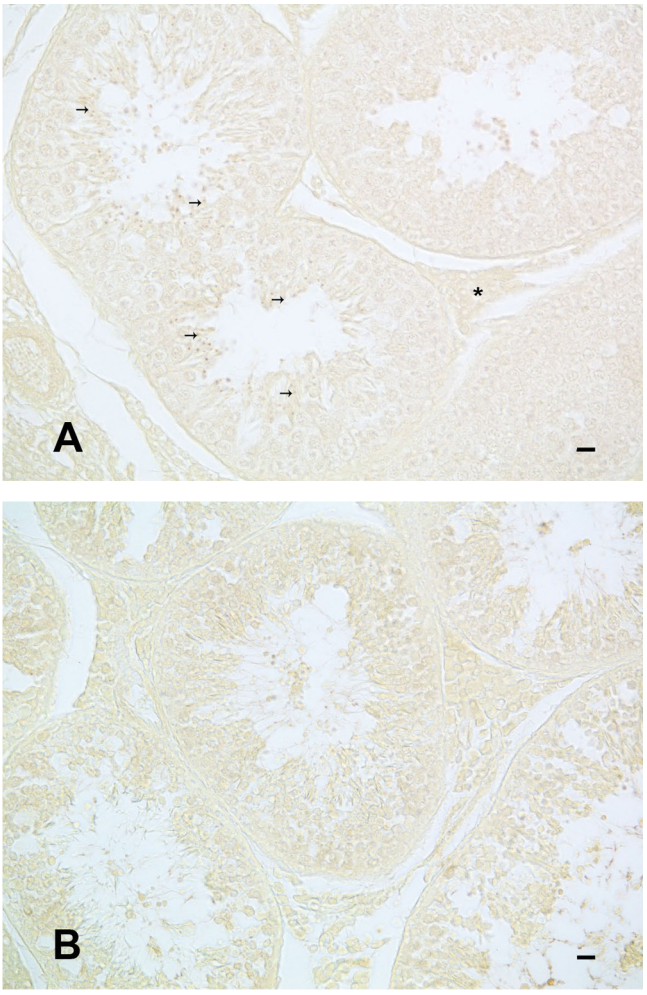

Fig. 2. (A) Numerous OX_1R immunopositive elongated spermatids (arrows); negative interstitial compartment (asterisk). (B) Negative control. Bars = 30 μm.

In particular, it has been demonstrated that OXA in rat testicular explants suppress the expression levels of several key Sertoli cells genes, such as those encoding Müllerian-inhibiting substance (MIS), inhibitor of testosterone secretion and controller of adult-type Leydig cells proliferation, and stem cell factor (SCF), the major paracrine stimulator of germ cells development (Barreiro et al., 2005). In line with this, OXA was capable of significantly inhibiting DNA synthesis at specific stages of the seminiferous epithelial cycle (Barreiro et al., 2005) and stimulating, in a dose-dependent manner, testosterone secretion by rat testicular tissue *in vivo* and *in vitro* (Barreiro et al., 2004).

Considering the presence of OX_1R in the tubular compartment of boar testis, we could also hypothesize a possible function of OXA on male germ cells cycle in pig. Moreover, we could suggest a potential action of OXA on the pig gonadotrope axis, similarly to that known for the corticotrope axis. Other studies have reported that OXA modulates adrenal steroidogenesis in the pig (Nanmoku et al., 2002). Further investigations, involving functional and ultrastructural analysis, may help us determine the role of the orexins system in the boar genital tract.

References

Assisi L, Tafuri S, Liguori G, Paino S, Pavone LM, Staiano N, et al. Expression and role of receptor 1 for orexins in seminiferous tubules of rat testis. Cell Tissue Res 2012;348:601–7.

Barreiro ML, Pineda R, Navarro VM, Lopez M, Suominen JS, Pinella L, et al. Orexin 1 receptor messenger ribonucleic acid expression and stimulation of testosterone secretion by orexin-A in rat testis. Endocrinology 2004;145:2297–306.

Barreiro ML, Pineda R, Gaytan F, Archanco MA, Burrell MA, Castellano JM, et al. Pattern of orexin expression and direct biological actions of orexin-A in rat testis. Endocrinology 2005;146:5164–75.

Dall'Aglio C, Pascucci L, Mercati F, Giontella A, Pedini V, Ceccarelli P. Immunohistochemical identification and localization of orexin A and orexin type 2 receptor in the horse gastrointestinal tract. Res Vet Sci 2009;86:189–93.

Dall'Aglio C, Pascucci L, Mercati F, Boiti C, Ceccarelli P. Localization of the orexin system in the gastrointestinal tract of fallow deer. Acta Histochem 2012;114:74–8.

de Lecea L, Kilduff TS, Peyron C, Gao X-B, Foye PE, Danielson PE. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci U S A 1998;95:322–7.

Ehström M, Gustafsson T, Finn A, Kirchgessner A, Gryback P, Jacobsson H, et al. Inhibitory effect of exogenous orexin A on gastric emptying, plasma leptin, and distribution of orexin and orexin receptors in the gut and pancreas in man. J Clin Endocrinol Metab 2005;90:2370–7.

Gulia KK, Mallick HN, Kumar VM. Orexin A (hypocretin-1) application at the medial preoptic area potentiates male sexual behaviour in rats. Neurosci Lett 2002;116:921–3.

Johren O, Neidert SJ, Kunner M, Dendorjer A, Dininiak P. Prepro-orexin and orexin receptor mRNAs are differentially expressed in peripheral tissues of male and female. Endocrinology 2001;142:3324–31.

Karteris E, Chen J, Randeva HS. Expression of human prepro-orexin and signalling characteristics of orexin receptors in the male reproductive system. J Clin Endocrinol Metab 2004;89:1957–62.

Kirchgessner AL, Liu M. Orexin synthesis and response in the gut. Neuron 1999;24:941–51.

Kohsaka A, Watanobe H, Kakizaki Y, Suda T, Shioth HB. A significant participation of orexin A, a potent orexigenic peptide, in the preovulatory luteinizing hormone and prolactin surges in the rat. Brain Res 2001;898:166–70.

Kuru M, Ueta Y, Serino R, Nakazato M, Yamamoto Y, Shibuya I, et al. Centrally administered orexin/hypocretin activates HPA axis in rats. Neuroreport 2000;11:1977–80.

Liguori G, Assisi L, Squillacioti C, Paino S, Mirabella N, Vittoria A. Presence, distribution and steroidogenic effect of the peptides orexin A and receptor 1 for orexins in the testis of the South American camelid Alpaca (Vicugna pacos). Gen Comp Endocrinol 2013;179:137–42.

Nanmoku Y, Isobe K, Sakurai T, Yamanaka A, Takekoshi K, Kawakami Y, et al. Effects of orexin on cultured porcine adrenal medullary and cortex cells. Regul Pept 2002;104:125–30.

Nurmio M, Tena-Sempere M, Toppari J. Orexins and the regulation of the hypothalamic–pituitary testicular axis. Acta Physiol 2010;198:349–54.

Ohkubo T, Tsukada A, Shamoto K. cDNA cloning of chicken orexin receptor and tissue distribution: sexually dimorphic expression in chicken gonads. J Mol Endocrinol 2003;31:499–508.

Overeem S, Kok SW, Lammers GJ, Vein AA, Frolich M, Meinders AE, et al. Somatotropic axis in hypocretin-deficient narcoleptic humans: altered circadian distribution of GH-secretory events. Am J Physiol Endocrinol Metab 2003;284:E641–7.

Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000;6:991–7.

Piper DC, Upton N, Smith MI, Hunter AJ. The novel brain neuropeptide, orexin-A modulates the sleep–wake cycle of rats. Eur J Neurosci 2000;12:726–30.

Russell SH, Small CJ, Kennedy AR, Stanley SA, Seth A, Murphy KG, et al. Orexin A interactions in the hypothalamo-pituitary gonadal axis. Endocrinology 2001;142:5294–302.

Russo F, Pavone LM, Tafuri S, Avallone L, Staiano N, Vittoria A. Expression of orexin A and its receptor 1 in the bovine urethroprostatic complex. Anat Rec 2008;291:169–74.

Sakurai T, Amameya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behaviour. Cell 1998;92:573–85.

Takahashi K, Arihara Z, Suzuki T, Sone M, Kikuchi K, Sasano H, et al. Expression of orexin-A and orexin receptors in the kidney and the presence of orexin-A-like immunoreactivity in human urine. Peptides 2005;27:871–7.

Zhang S, Blache D, Vercoe PE, Adam CL, Blackberry MA, Findlay PA, et al. Expression of orexin receptors in the brain and peripheral tissues of the male sheep. Regul Pept 2005;124:81–7.