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# Presence of orexin A and orexin 1 receptor in the buffalo prostate

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**ABSTRACT** - The orexins A and B are two peptides discovered initially in the rat lateral hypothalamus involved in the regulation of some body functions such as food intake, sleep/wake cycle, arterial pressure and heart rate. They interact with two receptors defined "orexin receptors 1 and 2", the first of which shows high selectivity for orexin A and the second binds both the peptides. In this preliminary study the presence of orexin A and orexin 1 receptor in the prostate of the buffalo *Bubalus bubalis* has been described utilizing the immunohistochemical avidin-biotin technique. The orexin A- and orexin 1 receptor- positive prostatic cells are very numerous and belong to the normal exocrine cytotype which makes up the glandular parenchyma. The role played by orexin A in the genital tract is poorly known. The peptide is retained to stimulate testosterone secretion and to inhibit spermatogonia proliferation in the rat testis.

**Key words:** Orexin A, Orexin 1 receptor, Prostate, Buffalo.

**INTRODUCTION** - Orexin A (oxA) and orexin B (oxB) are two peptides discovered in 1998 in the so called "feeding center" of the rat lateral hypothalamus. The orexins derive from the same precursor molecule, prepro-orexin, and bind two receptors defined orexin 1 receptor (ox1r) and orexin 2 receptor (ox2r). The first is more selective for oxA, while the second shows similar affinity for both substances. The orexins are retained to be involved in the regulation of central and peripheral functions of the body among which food intake (Sakurai et al., 1998), sleep/ wake cycle (Piper et al., 2000), gut motility (Voisin et al., 2003) and Leydig cell steroidogenesis (Barreiro et al., 2005). Moreover the injection of oxA in the medial preoptic area causes improvement of sexual performances in rats (Gulia et al., 2002). In this report we describe the presence of oxA and ox1r in the buffalo prostate by means of an immunohistochemical technique.

**MATERIAL AND METHODS** - The prostates of five adult buffaloes were collected in a local slaughterhouse soon after their death. Samples were fixed in Bouin's fluid, embedded in paraplast and routinely cut at 7  $\mu$ m. The sections were stained by the avidin-biotin immunohistochemical technique using, in the specific step, polyclonal antibodies raised in goat against oxA (Santa Cruz Biotechnology, sc-8070) and ox1r (Santa Cruz Biotechnology,

sc-8072). Both antisera were diluted 1:200 and incubated on sections overnight at 4°C. Before staining the sections were, sometimes, submitted to an antigen unmasking procedure by dipping them in 0.01 sodium citrate buffer, pH 6.0, and heating in a microwave oven for 10 min at 750W. The final staining was performed using 3-3' diaminobenzidine. Negative controls were obtained by substituting the specific antisera with phosphate buffer saline or by absorbing each of them with an excess of the relative peptide. The preparations were observed by a Nikon E 600 light microscope and photomicrographs were taken using a Coolpix 8400 Nikon digital camera.

**RESULTS AND CONCLUSIONS** - As in other ruminants, the buffalo prostate is composed of two parts: the body, smaller and transversally applied on the urinary bladder neck, and the disseminated portion, larger and completely contained in the lamina propria of the postcollicular segment of the urethra. We studied the second to which is referred the following description. OxA- and ox1r- immunopositive material is present in a conspicuous number of exocrine cells of the gland. Such cells are scattered in adjacent acini and show a focal distribution in the glandular parenchyma being grouped in clusters composed of dozens of elements, separated by zones lacking of any positivity. The immunoreactive cells have a basally located nucleus and a finely granular cytoplasm showing different intensity of staining. The role played by oxA in the prostate is, at present, completely unknown. Recently published data reports that in the adrenal gland of another ruminant species, the cattle, the peptide stimulates the production of catecholamines through the protein kinase C-mediated tyrosine hydroxylase activation (Kawada et al., 2003). The main source of oxA in the genital tract is the testis in which the presence of the peptide has been described in Leydig cells and spermatocytes and its function related to steroidogenesis and germ cell proliferation (Barreiro et al., 2005).

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