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Short communication

Immunohistochemical localization of orexin A and orexin type 2 receptor-positive cells in the placenta of dogs

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ABSTRACT

The aim of the present study was to examine the presence and distribution of cells that express immunopositivity for orexin A (OXA) and its type 2 receptor (OX2R) in the dog placenta toward the end of pregnancy using immunohistochemical techniques. In the placental fetal portion, a few OXA and OX2R-positive cells were seen scattered in the outermost coating layer of chorionic villi and in the trophoblastic protrusions. Closer to the maternal portion, immunopositive labeling for both peptides was visible in the glandular epithelia and that for OXA also in the endothelium of the capillaries. These observations allow us to hypothesize that the canine placenta may be not only a source of orexin A, but also its target, and that orexin A may play an important role in controlling the function of this important organ for normal fetal development.

Introduction

Orexin A and B are two peptides whose biological action is mediated by two specific receptors, OX1R and OX2R. Recent investigations have shown that the two peptides, derived from proteolysis of a common precursor, are at least 50% homologous. The orexin A (OXA) sequence is fully preserved among a large variety of mammals (Sakurai et al., 1998) and its binding affinity is the same for both receptor types (Smart and Jerman, 2002). The orexins and their receptors constitute the family of the orexinergic factors, which have attracted increasing interest in recent years because of their wide distribution in the body. Early studies showed the presence of orexins in the hypothalamic neurons of the rat (Sakurai et al., 1998) and their role in food intake (Kukkonen et al., 2002), however, many other studies demonstrated their production in different peripheral tissues and cell types that are either indirectly linked to appetite, or not linked at all. In particular, their production was shown in the gastrointestinal tract (Dall'Aglio et al., 2008, 2009), in some exocrine and endocrine glands, including salivary glands (Dall'Aglio et al., 2011; Leone et al., 2012), pancreas (Dall'Aglio et al., 2010) and adrenal glands (Randeva et al., 2001), and in organs of the male and female genital tract (Russo et al., 2008; Pavone et al., 2009; Tafuri et al., 2010).

Among the various peripheral organs where orexins have been found, OXA was visualized in syncytiotrophoblastic and decidual cells of the human placenta (Nakabayashi et al., 2003). The functional role of this presence has not yet been determined, but it is hypothesized that OXA presumably reaches the fetus through the circulation, hence affecting fetal growth and the outcome of the pregnancy.

More recently, the presence of OXA and its receptors was also detected in the feline placenta at the end of pregnancy (Dall'Aglio et al., 2012). This study confirmed what has been shown in humans (Nakabayashi et al., 2003), and has extended our knowledge about the distribution of orexins in domestic animals and highlights their importance in the outcome of pregnancy.

In order to gain further knowledge about the distribution of orexins in the placenta we examined the immunohistochemical distribution of OXA and OX2R in the placenta of the dog toward the end of pregnancy.

Materials and methods

Six mixed-breed dogs at approximately 55–60 days of gestation were used in the study. The dogs, admitted to the day-hospital service provided by the Veterinary Teaching Hospital of the University of Perugia, were given a routine clinical examination and pregnancy was diagnosed by two-dimensional ultrasonography

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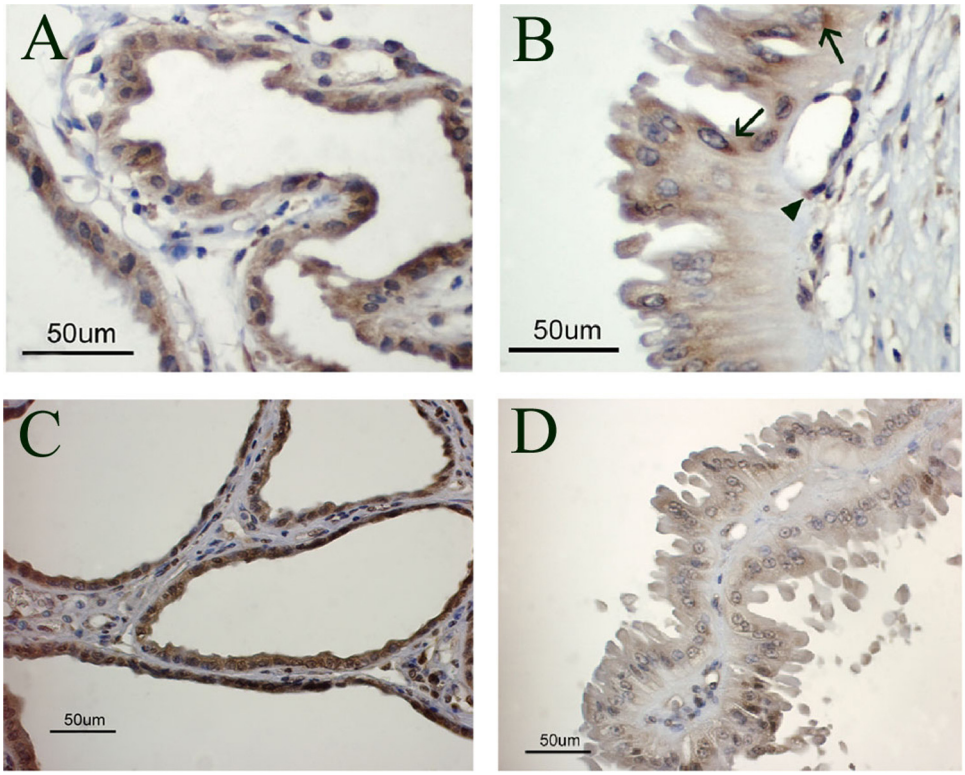

Fig. 1. Orexin system immunopositivity in the glands of the canine placenta. (A) OXA positivity in the deep uterine glands; (B) OXA positivity in the glandular chamber (arrows show perinuclear immunostaining of epithelial cells); arrowhead shows immunostaining of endothelial cells; (C) OX2R positivity in the deep uterine glands; (D) OX2R positivity in the glandular chamber.

in combination with color Doppler and PW Doppler examination (Medison Sonoace 8800 Full Digital, Austria) using a microconvex probe of 6.5 MHz (Di Salvo et al., 2006). The weeks of gestations were calculated on the basis of fetal crown-rump length (Kutzler et al., 2003). According to the clinical history provided by their owners, none of the animals had any pregnancy complications. Next, with the written consent of their owners, the animals were subjected to ovario-hysterectomy by spaying. Surgical procedures were carried out under general anesthesia and all bitches were premedicated with medetomidine (Sedator® Ati srl azienda terap, Italy) at a dose of 10 μg/kg and butorfanol (Dolorex® Intervet Italia srl) at a dose of 0.2 mg/kg. General anesthesia was induced with approximately 4–6 mg/kg of propofol (Rapinovet® Intervet Italia Srl) and maintained with isoflurane vaporized in oxygen. After three days of hospitalization, the dogs were returned to the kennel.

Immediately upon removal of the pregnant uteri, placental samples were promptly fixed by immersion in 4% formaldehyde solution and subsequently processed for embedding in paraffin wax. The immunohistochemical reaction sites were visualized using the following reagents: normal goat serum (1:10; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-OXA mouse monoclonal antibody (1:100; R&D Systems, Minneapolis, MN, USA), anti-OX2R rabbit polyclonal antibodies (1:100, Chemicon, Temecula, CA, USA), biotinylated goat anti-mouse IgG, biotinylated goat anti rabbit IgG, avidin-biotin-complex and diaminobenzidine (DAB) as the chromogen.

Briefly, serial 5 μm thick-sections were dewaxed and then microwaved for 15 min in 10 mM citric acid (pH 6.0) for antigen retrieval. All subsequent steps were carried out in a moist chamber at room temperature. To prevent non-specific binding of the two primary antibodies, after proper cooling, the sections were pre-incubated for 30 min with normal goat serum. Subsequently, serial sections were incubated overnight, each with one of the corresponding primary antibody.

The next day, after washing in PBS, the sections were incubated for 30 min at room temperature with the specific secondary biotin-conjugated antibody and then processed for 30 min using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA). Finally, the tissue sections were repeatedly rinsed with PBS and visualized with DAB solution. At the end of the immunoreaction, the sections were counterstained with hematoxylin, dehydrated and mounted in Canada Balsam Natural (BDH, Poole, Dorset, UK).

Sections in which the primary antibodies were omitted were used as a control of non-specific staining (data not shown). The cells were considered positive for OXA and its receptor only when cytoplasmic staining was evident, independently of its immunointensity.

Results

The immunohistochemical reactions revealed the presence of cells showing cytoplasmic positive reaction for OXA and OX2R: in most cases both the positive immunoreactivities were co-expressed by the same cells. In particular, positive immunoreaction for OXA and OX2R was detectable in the cuboidal epithelium of the deep uterine glands (Fig. 1A and C, respectively) and in the high columnar epithelial cells of the glandular chambers (Fig. 1B and D, respectively). In the high epithelium, the positivity was localized near the nucleus and in the apical cytoplasmic portion (Fig. 1B, arrows) while, in the cuboidal cells, it was uniformly distributed. Endothelial cells of the capillaries near glands showed OXA- but not OX2R-positivity (Fig. 1B, arrowhead). Moving further toward the fetal portion of the placenta, the immunopositivity for OXA and OX2R was seen in the epithelial cells of the trophoblastic