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Immunohistochemical detection of the orexin system in the placenta of cats

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ABSTRACT

The aim of the present study was to investigate the presence and distribution of cells containing orexin A (OXA), and orexin type 1 and 2 receptors (OX1R and OX2R, respectively) in the feline placenta by means of immunohistochemical technique. OXA was identified in several decidual and syncytiotrophoblastic cells present in the lamellar portion of the placenta. In the same placental structures, few decidual and syncytiotrophoblastic cells showed the presence of OX1R-like immunoreactivity. Characteristically, immunopositivity for OX2R, but not for OX1R, was evidenced in the cells of the glandular layer. The orexinic system was not expressed in the uterine structures that were not engaged by the chorion. Our results provide the first evidence of the presence of a placental orexinic system in a mammalian species. Orexin A and both OX1R and OX2R are unequally distributed within the cat placenta. Local OXA production and the presence of specific receptors, differentially expressed in the placental structures of the cat, suggest that the orexinic system may participate in placental growth and development as well as in the regulation of its steroidogenic capacity via endocrine, paracrine and/or autocrine mechanisms.

1. Introduction

The orexin family consists of two separate peptides, orexin-A (OXA) and orexin-B (OXB), derived from proteolysis of a common 130-amino acid precursor, prepro-orexin. Interestingly, OXA and OXB share 40% homology, and the sequence of OXA is unvaried among rat, human, mouse, pig and cow (Sakurai et al., 1998). The biological actions of these peptides are mediated by two specific G protein-coupled receptors, OX1R and OX2R. OX1R binds selectively to OXA, whereas OX2R binds to both OXA and OXB: the latter has 100–1000 fold greater affinity for OX2R than for OX1R (de Lecea et al., 1998; Sakurai et al., 1998). Orexins were initially identified in neurones of the dorsal and lateral hypothalamic areas (de Lecea et al., 1998; Sakurai et al., 1998) where they are involved in the control of feeding behaviour and energy balance (Sutcliffe and de Lecea, 1999) as well as in sleep, blood pressure and body temperature regulation through projections to multiple neuronal systems (Peyron et al., 1998). Subsequently, orexins and/or their cognate receptors were recognized outside the central nervous system in different peripheral tissues and cell types including those of the gastrointestinal tract, endocrine pancreas, adrenal gland,kidney, ovary and testis (Ehrström et al., 2005; Nakabayashi et al., 2003; Näslund et al., 2002; Dall'Aglio et al., 2008, 2009, 2010; Russo et al., 2008; Pavone et al., 2009). The wide distribution of the orexinic system outside the central and peripheral nervous system suggests that orexins exert a pleiotropic role in regulating-diverse physiological functions with mechanisms that, however, remain to be disclosed (Heinonen et al., 2008).

Recently, OXA was detected also in syncytiotrophoblasts and decidual cells of the human placenta (Nakabayashi et al., 2003), but little is known about its biological significance in the function of the foetal–placental unit. It has been hypothesised that OXA produced in the placenta could reach the foetus via the circulation and hence affect foetus growth (Nakabayashi et al., 2003). As far as we know, however, there is no evidence of the presence and distribution of orexin producing cells in the placentas of domestic mammals which, moreover, are characterized by different numbers and barrier structures between maternal and foetal circulation. Furthermore, there is no proof of the expression of orexin receptors within the placental tissue. Thus, the purpose of this study was to investigate the presence of the orexinic system in the endotheliochorial placenta of cats. In addition, precise cell localization of both OXA and its cognate receptors within the different placental structures of cats that have the capability of synthesising steroid hormones (Ito et al., 1987) could provide further information on the target of the orexinic system and its mechanism of action, either endocrine, paracrine or autocrine.

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2. Materials and methods

2.1. Animals

Uterine and placental tissues were collected from six mixed-breed cats at approximately 55–60 days of gestation. The cats, registered in the day-hospital service provided by the Veterinary Teaching Hospital, underwent ovario–hysterectomy for spaying with the written consensus of their owners. Following routine clinical examination, pregnancy was diagnosed by two-dimensional ultrasonography in combination with colour Doppler and PW Doppler examination (SONOACE 8800 Full Digital Medison Inc., Austria) using a microconvex probe of 6.5 MHz (Scotti et al., 2008). The weeks of gestations were calculated on the basis of crown-ramp length (Evans and Sack, 1973). None of the animals had any pregnancy complication, according to the clinical history provided by their owners. Surgical procedures were carried out under general anaesthesia induced by an association with Domitor (80 μg/kg) and Ketamine (5 mg/kg). After three days of hospitalization, the cats were returned to the kennel; no animal was killed for this study.

2.2. Tissue preparation and immunohistochemistry

Immediately upon removal of the pregnant uteri, two transverse slices were excised from each cat, one from the anti-mesometrial area of a placental girdle and the other from an area not involved in placentation. Tissue samples, each approximately 0.5 cm wide, were promptly fixed by immersion in 4% formaldehyde solution in PBS (0.1 M, pH 7.4) at room temperature for 24 h and subsequently processed for embedding in paraffin. Routine tissue preparation procedures for the immunohistochemical detection of the target proteins of interest were performed. The immunohistochemical reaction sites were visualized using the avidin–biotin-complex (ABC Elite Kit, Vector Laboratories, Burlingame, CA, USA) and diaminobenzidine–nickel solution (Vector Laboratories) 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 primary antibodies, after proper cooling, the sections were pre-incubated for 30 min with normal goat serum (1:10; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Subsequently, serial sections were incubated overnight, each with one of the following primary antibodies: anti-OXA mouse monoclonal antibody (1:100; R&D Systems, from R&D Systems Inc., MN, USA), anti-OX1R and anti-OX2R rabbit polyclonal antibodies (1:100; Sigma, Sigma–Aldrich, MO, USA, and Chemicon, from Millipore Corporate Headquarters, MA, USA, respectively).

The next day, after washing in PBS, the sections were incubated for 30 min at room temperature with the specific secondary biotin-conjugated antibody, goat anti-mouse IgG and goat anti rabbit IgG (1:200, Vector Laboratories, Burlingame, CA, USA and Zymed, San Francisco, CA, USA, respectively), and then processed for 30 min using the Vectastain ABC kit (Vector Laboratories). Finally, the tissue sections were repeatedly rinsed with PBS and developed with DAB solution; therefore, all the immunopositive cells were stained brown. After several rinses in PBS, the sections were dehydrated and mounted in Canada Balsam Natural (from BDH, Poole, Dorset, England).

Sections in which the primary antibodies were omitted or substituted with pre-immune gamma globulin were used as a control of unspecific staining. The cells were considered positive for OXA and its receptors only when cytoplasmic staining was evident, independently of its immunointensity. All tissue analyses were carried out on coded slides using a light microscope (Nikon Eclipse E800) connected to a digital camera (Dxm 1200 Nikon digital camera). Some sections were processed for the purpose of a morphological study using the trichromic Gabe and Martoja stain (Gabe and Martoja-Pierson, 1957).

3. Results

The decidual cells were generally voluminous, roundish or polyhedric, with cytoplasm rich in granules whereas syncytiotrophoblasts, derived from fused trophoblasts, were multinucleated; both were present in the lamellae (L) of the placental labyrinth (Fig. 1). Positive immunoreaction (IR) for OXA was detectable in several cells showing the typical morphological features of decidual and syncytiotrophoblastic cells (Fig. 2a). Characteristically, OXA immunoreactivity was localized around the nucleus while it was substantially absent near the cytoplasmic membrane (Fig. 2a). Some decidual and syncytiotrophoblastic OXA immunoreactive cells were localized near the capillary vessels (C) into which they likely secrete their content as shown in Fig. 2b.

Immuno staining for OX1R was observed in a few of the decidual and syncytiotrophoblastic cells scattered in the labyrinthic portion of the placenta (Fig. 3a). Positive IR for OX2R was evidenced only in the cells forming the glandular portion of the uterus (Fig. 3b).

No other histological structure in the uterus outside the placental girdle reacted positively to OXA or to its cognate receptors. Staining for OXA, OX1R, and OX2R was completely absent when the primary antibody was substituted with non-immune serum (Fig. 4).

4. Discussion and conclusions

The feline placenta is classified as a zonary placenta of the endotheliochorial type, due to the distribution of the chorial villi and the histological organization of the interhemal barriers (materno-foetal barrier) (Miglino et al., 2006). In the cat, the placenta has a trabecular, villous structure consisting of a lamellar zone with elongated, parallel foetal (chorionic) and maternal lamellae, a junctional zone where foetal and maternal tissues closely interface each other, and a zone of endometrial glands (Amoroso, 1952; Leiser and Koob, 1993). Once formed, the placenta develops into a labyrinth-like structure with a network of trophoblast cells

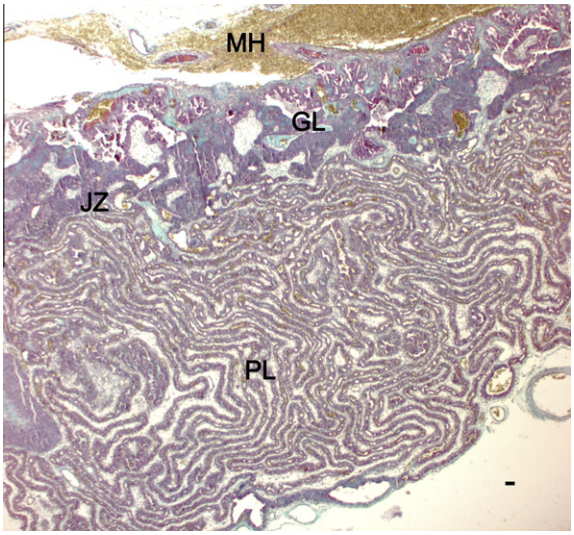

Fig. 1. Light microphotograph of the placenta of a pregnant cat at approximately 58 days of gestation. (PL = placental labyrinth, JZ = junctional zone, GL = glandular layer, MH = marginal hematoma). Scale bars = 40 μm.