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Distribution of olfactory marker protein in the rat vomeronasal organ

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ABSTRACT

Olfactory marker protein (OMP) may act as a modulator within the olfactory signal-transduction cascade. It has also been shown to have some importance in development of olfactory sensory organs. Here we used high resolution immunocytochemistry to localize OMP in the rat vomeronasal organ (VNO). Immunofluorescence for OMP was abundant in cilia and in apical dendrites of sensory cells, mostly associated with intraepithelial capillaries. Perikarya were stained to a lesser extent while intense OMP immunoreactivity was seen in axons of sensory neurons. Single cells within the non-sensory portion of the VNO exhibited intense OMP immunofluorescence in apical cilia and weak cytoplasmic staining. Some of the exocrine cells in the vomeronasal glands contained OMP positive secretory granules. Electron microscopy revealed that non-sensory ciliated cells had short rod like kinocilia as well as microvilli. These cells contained secretory vesicles. Their basal portion was in close apposition to nerve endings. Our findings suggest that the sensory part of the VNO contains OMP positive sensory neurons and that the non-sensory epithelium may contain secondary sensory cells. In addition OMP may be liberated from secretory glands into vomeronasal secretions.

1. Introduction

Olfactory marker protein (OMP) is a 19 kDa protein that is known to specifically occur in olfactory sensory neurons (Bock et al., 2009; Farbman and Margolis, 1980; Fleischer et al., 2006; Graziadei et al., 1980; Margolis, 1972; Wensley et al., 1995). OMP is widely used as a molecular marker for olfactory neurons in both the olfactory mucosa and the sensory portion of the vomeronasal organ (VNO) in different species (Barrios et al., 2014a,b; Berghard et al., 1996; Sasuga et al., 2013; Smith et al., 2011). The functional properties of OMP are still a matter of controversy. It has been shown to play a critical role in olfaction (Buiakova et al., 1996). OMP may also have some importance for postnatal development of mother preference (Lee et al., 2011), for maturation of olfactory neurons and for the level of selectivity in stimulus-response (Buiakova et al., 1996). Recent observations indicate that OMP is not confined to olfactory neurons since it has been described also in a few tissues unrelated to the olfactory system (Kang et al., 2015).

The vomeronasal duct forms the center of the VNO. Its medial portion is lined by the sensory epithelium (SE) which contains sensory neurons, basal cells, supporting cells, sustentacular cells (Barrios et al., 2014a) and intraepithelial capillaries (Breipohl et al., 1981). Sensory neurons extend their olfactory cilia into the lumen. They are surrounded by cells which have longer microvilli (Hofer et al., 2000; Dennis et al., 2003). Laterally the duct is lined by 'non-sensory epithelium' (NSE) characterized by four cell types: Dark, light and pale non-ciliated cells, as well as ciliated columnar cells (Elgayar et al., 2014). The NSE is closely associated with one or two large vomeronasal veins (Doving and Trotier, 1998). Vomeronasal glands are located in the submucosal layer of the NSE, their excretory ducts feed into the vomeronasal duct (Adams and Wiekamp,1984; Doving and Trotier 1998).

In most mammals the VNO is known to play an important role in chemical communication (Achiraman et al., 2010; Barrios et al., 2014b; Jacobson et al., 1998). It is likely to be the primary target of pheromones thus triggering social behaviors including sexual arousal and stress response (Clancy et al., 1984; Holy et al., 2000; Leypold et al., 2002; Luo and Katz, 2004). The underlying molecular mechanisms are still far from being understood. Recently we observed the expression of sex hormone binding globulin and of corticosteroid binding globulin in the rat olfactory epithelium, in the VNO and in the olfactory bulbs (Dolz et al., 2013; Ploss et al., 2014a,b).

The current immunohistochemical study seeks to examine the distribution of OMP in the rat VNO in greater detail. Electron

Abbreviations: NSE, non-sensory epithelium; OMP, olfactory marker protein; SE, Sensory epithelium; VNO, Vomeronasal organ.

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microscopy was employed to study ultrastructural features of the various cell types in SE and NSE.

2. Materials and methods

2.1. Tissue preparation

All animal experiments were performed in accordance with German law. Protocol was approved by Thuringian state government (# 02-040/40). Tissue preparation was performed as described before (Ploss et al., 2014b). Briefly, wistar rats (adult males n = 3, adult females n = 2) were killed by prolonged ether anesthesia followed by cardiac perfusion with 4% paraformaldehyde in PBS (0,1 M sodium phosphate buffer pH 7,2, containing 0,9% NaCl). Tissue samples containing the VNO were rapidly dissected and postfixed in the same fixative prior to dehydration through ascending ethanol series and embedded in EPON 812 (Ferak, Berlin, Germany). 1 μm serial sections were cut on a Reichert Ultracut microtome, collected on APES-coated glass slides (Thermo Scientific, Braunschweig, Germany) and air dried.

2.2. Immunohistochemistry

For immunohistochemistry epoxy resin was removed by incubation in 10% sodium methoxide, methanol/benzene 1:1 and 2× in acetone 2 min each. Thereafter sections were rinsed in PBS. To block non-specific reactions the sections were preincubated in 2% normal donkey serum diluted in PBS at room temperature (RT) for 30 min. After washing in PBS sections were incubated with goat anti-OMP (Wako chemicals, USA) diluted 1:500 in PBS in a humid chamber at 4 °C overnight. Controls were performed with normal goat serum instead of the specific antibody. After rinsing in PBS sections were incubated in the dark with donkey Alexa Fluor 568 labeled anti-goat IgG (Invitrogen, Karlsruhe, Germany) diluted 1:200 in PBS at RT for 60 min. After washing in PBS sections were incubated in the dark with nuclear stain (Hoechst) diluted 1:1000 in PBS at RT for 15 min. For immunoperoxidase staining we incubated sections that had been treated with the anti OMP as described above with anti-goat IgG (Sigma Aldrich, Munich, Germany) 1:200 in PBS at RT for 60 min. After washing in PBS sections were incubated with Peroxidase-anti-peroxidase complex (goat-PAP, Sigma Aldrich, Munich, Germany) diluted 1:200 in PBS at RT for 60 min. Immunoprecipitates were stained with DAB and H_2O_2 for 5 min (FAST kit, Sigma Aldrich, Munich, Germany). Stained sections were rinsed in PBS and mounted with either aqueous mounting medium Mowiol® (AppliChem, Darmstadt, Germany) or, after dehydration through ascending ethanol series and clearing in xylene, with Entellan® (Merck, Darmstadt, Germany). Analysis of the sections was performed with an Olympus BX 50 microscope equipped with epifluorescence illumination (filter combinations WU and WG) and with interference contrast optics. An Olympus DP10 camera was used for microphotography.

2.3. Electron microscopy

Tissue samples containing the VNO were obtained from perfusion fixed rats as described above. After repeated rinsing in 0.1 M cacodylate buffer specimen were fixed with 2% glutaraldehyde (0.1 M cacodylate buffer, pH 7.4, 5% sucrose) for 30 min at 20 °C. Subsequently tissue samples were rinsed in 0.1 M cacodylate buffer (pH 7.4, 6.8% sucrose) and postfixed with a freshly prepared mixture of 2% osmium tetroxide (in ddH_2O) and 3% potassium ferrocyanide (0.2 M cacodylate, pH 7.4) for 2 h at 4 °C. Thereafter specimen were washed thoroughly in 0.1 M cacodylate solution (pH 7.4) until the solution remained clear. (All chemical obtained from Sigma-Aldrich, Munich, Germany) Tissue samples were dehydrated in graded ethanol series and embedded in EPON 812 (Ferak, Berlin, Germany) via acetonitrile as intermedium. Tissue blocks were polymerized at 60 °C for one week. Ultrathin sections (70 nm) prepared with low angle diamond knives (DIATOME, Biel, Switzerland) were mounted on Formvar coated H9 SPEC Cu copper grids (Science Services, Munich, Germany) and stained with 1% uranyl acetate (in methanol) and freshly prepared lead citrate (25 mg/10 ml distilled water). Sections were examined with an EM 902 (ZEISS, Oberkochen, Germany) with an accelerating current of 80 KV.

3. Results

3.1. Sensory epithelium

Sensory cells of the VNO extended long dendrites which terminated in olfactory knobs and olfactory cilia. Dendrites were surrounded by supporting cells and by small capillaries. OMP immunostaining was found in olfactory cilia and in dendrites of sensory neurons (Fig. 1). The perinuclear cytoplasm of most of these cells showed OMP immunofluorescence (Fig. 2). Axons of sensory cells were seen in the submucosal layer, they were also specifically immunostained. Higher magnifications of the apical portion of the SE revealed that the superficial layer consisting mostly of supporting cell microvilli and of olfactory cilia contained the most intense OMP immunofluorescence. Some of the OMP positive dendrites were in close apposition to intraepithelial capillaries (Fig. 3). Sensory cell nuclei, cytoplasm of supporting cells and of basal cells were OMP-negative. The results showed no significant sex differences. Controls did not show any specific staining in SE.

3.2. 'Non-sensory' epithelium

The squamous epithelium lining the NSE consists of ciliated and non-ciliated cells interposed by scattered goblet cells. A small portion of these ciliated cells appeared to be narrow and cone shaped. They contained small OMP positive cytoplasmic granules

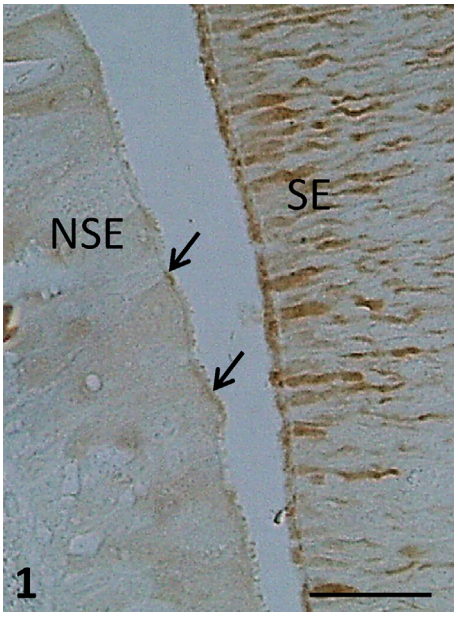

Fig. 1. Immunoperoxidase staining for OMP in the rat VNO. Apical dendrites of sensory cells within SE show specific immunoreactivity (brown precipitation). Cilia of single cells in the NSE are OMP positive (arrows). Scale bar = 10 μm.