

Functional Characterization of Olfactory Binding Proteins for Appeasing Compounds and Molecular Cloning in the Vomeronasal Organ of Pre-pubertal Pigs

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Abstract

The appeasing behaviour of pre-pubertal pigs appears to result from the perception of maternal odours (fatty acids) and of steroids coming from the male. We have used a ligand-oriented approach to functionally characterize olfactory binding proteins involved in the detection of appeasing compounds in the nasal mucosa (NM) and the vomeronasal organ (VNO) of pre-pubertal pigs. Several proteins were identified, combining binding assay, immunodetection and protein sequencing. Their sites of expression in nasal and vomeronasal tissues were studied by reverse transcription polymerase chain reaction (RT-PCR). The proteins belong to the lipocalin superfamily: Alpha-1-acid glycoprotein (AGP), odorant-binding protein (OBP), salivary lipocalin (SAL) and Von Ebner's gland protein (VEG), and displayed different binding capacities for the appeasing compounds. RT-PCR experiments showed that OBP and VEG are expressed not only in the NM, but also in the VNO and that SAL is only expressed in the VNO. This is the first report of the expression of these lipocalins in the VNO. Different binding affinities between lipocalins and appeasing compounds, together with their different localizations in the olfactory systems, suggest multiple possibilities for the peripheral coding of appeasing signals.

Key words: pig maternal pheromone, odorant-binding protein, Von Ebner's gland protein, salivary lipocalin, steroid

Introduction

Lipocalins constitute a heterogeneous family of small, secreted proteins that share amino acid motifs, a common structure and the ability to bind a remarkable array of small hydrophobic molecules (Akerstrom *et al.*, 2000). Among them, olfactory binding proteins mediate the reception of olfactory signals in several biological fluids and organs implicated in the chemical communication of mammals (Tegoni *et al.*, 2000). Their precise physiological role is partially understood and that led to an arbitrary classification, based on their known (or unknown) binding properties towards different classes of ligands: pheromone-binding proteins (PBPs) and odorant-binding proteins (OBPs) differ in their localizations (Pelosi, 2001). They are evolutionary and structurally unrelated to insect PBPs and OBPs, despite their common function of odorant binding (Pelosi, 1994).

Mammalian PBPs are secreted in diverse biological fluids involved in social and sexual behaviours mediated by pheromones (urine, vaginal discharge or saliva) such as rodent major urinary protein (MUP) (Finlayson *et al.*, 1965)

and aphrodisin (Singer *et al.*, 1986), or the salivary lipocalin (SAL) characterized in pig (Marchese *et al.*, 1998).

The physiological role of OBPs is less documented. They are secreted in the mucus lining the nasal cavity and, contrary to PBPs, bind a broad array of hydrophobic ligands with dissociation constants in the micromolecular range (Tegoni *et al.*, 2000). This apparent lack of binding specificity led authors to confer on OBPs the role of solubilization and transport of odorant molecules to their target receptors located in the membrane of olfactory receptor neurons (Pelosi, 2001). OBPs are also assumed to concentrate odorants and/or to scavenge them from receptors in a deactivation process (Pelosi, 2001). The poor binding specificity observed for OBPs could be explained by the fact that none of the ligands commonly used in published binding assays are relevant to the animal, i.e. their perception does not evoke any specific behaviour. This point is meanwhile of critical importance to study the involvement of OBPs in odour discrimination.