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Short Communication

Presence and distribution of leptin and its receptor in the minor salivary glands of the donkey

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ABSTRACT

Leptin is a hormone widely diffused in the mammalian body in which it plays functions that go far beyond control of appetite and energy metabolism. The finding that it is present in the major salivary glands of various animal species is of interest for the functional implications that it may imply.

Since there are no data on the presence of leptin and its receptor in the minor salivary glands, the aim of this study was to demonstrate their presence and distribution in such glands of donkeys. This latter was chosen as species of reference because the monogastric herbivore shows intense salivation during their masticatory activity.

Tissue samples were collected from four adult donkeys, of both sexes, following slaughter. Samples were fixed, embedded in paraffin, and processed for immunohistochemical analysis using primary antibodies directed against leptin and its receptor. Controls for non-specific staining were always included.

Leptin and its receptor were found in the minor salivary glands. Their distribution was similar to that described in the major salivary glands of animal species that have been investigated to date. We hypothesized that leptin can play a role in regulating gland function, via an autocrine/paracrine mechanism.

Introduction

The minor salivary glands consist of tubulo-acinar adenomeri, with mucous and serous cells, that conglomerate in lobules and a complex system of excretory ducts. Designated "minor glands" because they are less extensive than the major salivary glands, they are found in the "lamina propria" of the oral cavity wall or within the thickness of oral organs and are named according to their location. Their activity, qualitative and quantitative secretion are controlled by the vegetative nervous system through sympathetic and parasympathetic fibres.

In the major salivary glands of humans and different animal species many recent studies highlighted the presence and localization of several neuropeptides, like orexins and leptin (Bohlender et al., 2003; Leone et al., 2012; Dall'Aglio et al., 2011, 2012), which probably play a role in their functional control. Independently of the animal species, leptin was found in the serous cells of the gland adenomeri and in intra- and inter-lobular ductal epithelial cells. In the same glands, localization of the leptin receptor was restricted to ductal epithelial cells (Bohlender et al., 2003; Leone et al., 2012; Dall'Aglio et al., 2012; De Matteis et al., 2002).

Leptin is known to be involved in control of appetite, energy metabolism, cancer risk and mucosa epithelium renewal of the gastrointestinal tract (Schneider et al., 2001; FitzGerald et al., 2005; Schapher et al., 2011). Such observations and our findings that leptin and its receptor are colocalized in cells of the major salivary glands led us to retain that, at least in such glands, the hormone could have not only an endocrine modality of diffusion but also an autocrine/paracrine mechanism of action (Dall'Aglio et al., 2012). Detecting the leptin receptor, often in the same cells that produce leptin, is evidence in support of this hypothesis.

There are no data on the presence of leptin in the minor salivary glands despite their widespread distribution in the mouth and their contribution to saliva production. Consequently, the objective of this study was to fill the gap investigating the presence of leptin and its receptor in the minor salivary glands of the donkey, by means of an immunohistochemical technique. The donkey was chosen as reference species because a copious salivation notoriously occurs in the herbivorous during food mastication.

Materials and methods

Four healthy adult donkeys were used. Specimens of lingual, labial and buccal salivary glands were collected, immediately after

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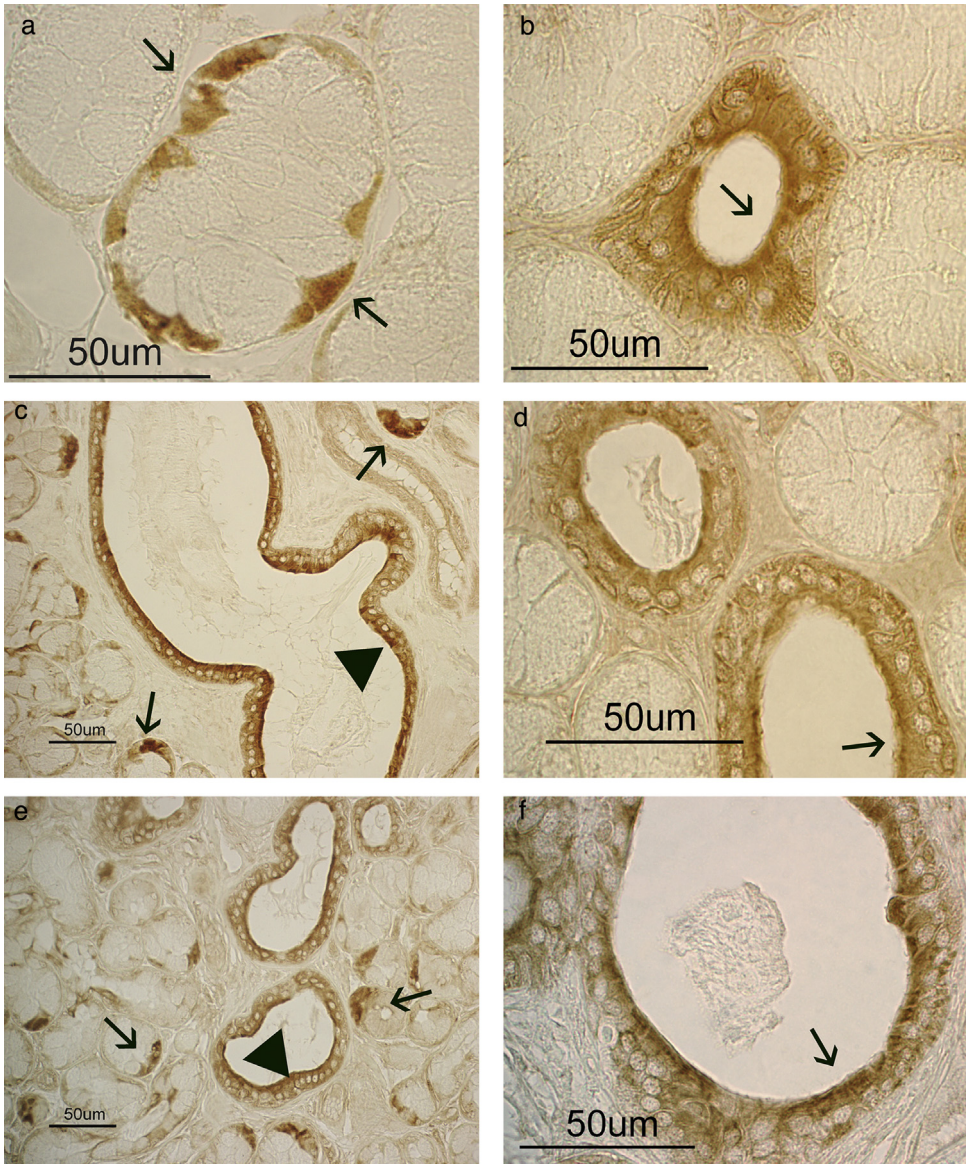

Fig. 1. Distribution patterns of leptin (panels a, c and e) and leptin receptor (panels b, d and f) immunopositivity in the minor salivary glands – positivities from buccal, labial and lingual glands are shown, respectively, in panels a/b, c/d and e/f. Arrows indicate leptin-positivity in serous cells (panels a, c and e) and leptin receptor-positivity (panels b, d and f). Arrowheads show leptin-positivity in duct epithelial cells (panels c and e).

slaughter, and fixed by immersion in 4% paraformaldehyde solution in 0.1 M PBS, pH 7.4, for 24 h at room temperature. Subsequently, such material was processed for embedding in paraffin. For the immunohistochemical reactions, samples were cut into 5 μm thick serial sections that were mounted on poly-L-lysine coated glass slides and dried overnight in a stove at 37 °C. The results of the immunoreaction were visualized, using the avidin-biotin-complex (ABC Vectastain Kit, PK-4000; Vector Laboratories, Burlingame, CA, USA), and the 3.3′-diaminobenzidine-4-HCl (DAB) as chromogen. The following primary antibodies were used: anti-Leptin rabbit polyclonal (sc-842, Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:100) and anti-Leptin Receptor goat polyclonal antibodies (sc-1834, Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:100). For the former, the specificity of the reaction in the equine species is indicated in the data sheet. For the latter, since specificity is not indicated by the manufacturer, despite its prior use in horses (Lange Consiglio et al., 2009; Dall'Aglio et al., 2013), we performed the immunoreaction pre-adsorbing the antibody with the respective blocking peptide (sc-1834P, Santa Cruz Biotechnology, Santa Cruz, CA, USA), according to the manufacturer's instructions. Controls for non-specific staining were always carried out using PBS instead of primary antibodies. The antibody used for the leptin-receptor localization is recommended for the detection of its short and long forms, as reported in the datasheet, and it recognizes the C-terminus of the short form of Ob-R of mouse origin.

In detail, dewaxed sections were microwaved three times, each for 5 min, in 10 mM citric acid (pH 6.0), for antigen retrieval. The citric acid solution was added after each cycle to prevent excessive evaporation. After cooling at room temperature for 20 min, to prevent non-specific primary antibody binding, the sections were incubated for 30 min with normal serum, obtained from the donor species of the secondary antibodies. To allow right antigen–antibody reactions and to prevent reagent evaporation and drying of the sections, all subsequent incubations were carried out in a humid chamber at room temperature. Overnight incubation with the primary antibody was followed by washing in phosphate-buffered saline solution (PBS, pH 7.2), and 30 min incubation with the secondary biotinylated antibodies, goat anti-rabbit