

Distribution and expression of leptin in major salivary glands of a hyperleptinemia animal model

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Abstract

Saliva production is mainly regulated by the autonomic nervous system (sympathetic and parasympathetic); however studies indicate a possible hormonal influence on the control of salivary secretion. This study aims to assess if the induction of increased levels of circulating leptin influence the immunohistochemical expression of leptin at the level of major salivary glands in Wistar rats. It was found that the expression, in qualitative terms, of leptin has been positive, being more evident in submandibular and sublingual glands, either in the acini or ducts. However, through this technique, no obvious differences between groups could be observed. The results suggest that circulating leptin levels may not affect the expression of this hormone in the major salivary glands. Keywords: Hyperleptinemia, leptin, salivary glands, Wistar rats.

INTRODUCTION

The major salivary glands include parotid, submandibular and sublingual glands. Saliva performs several functions: protection, digestion, chewing, talking, swallowing, lubrication and anti-microbial action [1]. Despite saliva production being mainly regulated by the autonomic nervous system, there are studies indicating hormonal influence [2].

The hormone leptin, the product of the Ob gene (obese) and derived cytokine, is produced mainly in adipose tissue and is found circulating in free form or bound to proteins [3]. This hormone acts at hypothalamus level signalling feelings of fullness and has an important role in regulating food intake and energy balance [4]. In circulation, leptin concentration is proportional to body fat and reflects the nutritional status of an individual [5]. Changes in saliva protein secretion and composition, according to satiety state, have been referred [6] and several authors have reported the expression and distribution of leptin in salivary glands of humans and other animal species, including rats [7-10].

The objective of this study is to evaluate whether the expression of leptin in the major salivary glands of rats is influenced by the experimental increase in circulating leptin concentrations.

MATERIAL AND METHODS

Twenty eight male Wistar rats were divided in three groups: control (not submitted to any surgical procedure) (N=8); "serum" (with mini-pumps Alzet filled with PBS) (N=12) and leptin (with mini-pumps Alzet with 0,2µg/mL in PBS). The mini-pumps allowed a constant delivery of leptin to circulation at a constant rate, for 7 days. At the

end, circulating leptin levels were: 99.5 ng/dL, 62.0 ng/dL and 257.4 ng/dL, for control, "serum" and leptin groups, respectively. After 7 days, animals were euthanized through inhalant anesthetic overdose followed by exsanguination. Right salivary glands were removed and processed by routine histological techniques. Sections were stained with H&E and immunohistochemistry was performed using the "labeled- (strept) avidin-biotin" (LAB-SA) UltraVision Detection System kit (Thermo Scientific, USA, ref TP-015-HD). Prior to incubation with the anti-leptin antibody (Santa Cruz, Ob (A-20), sc-842, 1:50, 4°C, overnight) the sections were heated for antigen retrieval, in citrate buffer (pH 6.0), at 98° C, for 20 minutes. As a negative control, a salivary gland section with the primary antibody replaced by PBS was used, whereas positive control was performed by using a breast cancer section [11]. Slides were observed under light microscope at 250X magnification and immunostaining was evaluated qualitatively according to the following scale: 0 (negative); + (weak); ++ (moderate); +++ (strong).

RESULTS

Evident immunostaining was observed for parotid, submandibular and sublingual glands, with no differences between leptin and "serum" groups (Table 1). Higher intensity of staining, both in acini and ducts, was observed for submandibular glands, comparatively to sublingual and parotid. A variable amount of adipocytes in the parenchyma of the glands with positive immunostaining was also found.

Goups	Sublingual		Submandibular		Parotid	
	Acini	Ducts	Acini	Ducts	Acini	Ducts
"Serum"	++	++	+++	+++	+	++
Leptin	++	++	+++	+++	+	++

* 0 = Negative; + = weak; ++ = moderate; +++ = strong.

Table 1 Immunostaining for leptin in the major salivary glands*

DISCUSSION AND CONCLUSIONS

As already stated several authors have reported the expression and distribution of leptin in salivary glands of humans and other animal species [7-10]. The absence of differences in the present study can be related to the fact that we only increased circulating levels of leptin, without having caused changes in other molecules influencing obesity [12]. Another hypothesis is that regulation of leptin in the salivary glands is not the same as in the hypothalamus. However, as the hypothalamus is directly involved in controlling energy balance [13], we can put the hypothesis that there is need for further adjustment for circulating leptin levels. Observation of immunostaining more evident in the submandibular and sublingual glands can mean that they are the salivary glands more involved in contributing for the leptin found in saliva, comparatively to parotid, at least in rodents.

Neves [14] observed changes in protein profile in the saliva of the animals used in this study, i.e. animals with hyperleptinemia. However, the levels of salivary leptin were not accessed. These results suggest that leptin is expressed at salivary gland level, mainly at submandibular acini and duct level, although circulating levels appears not affect its expression in the major salivary glands.

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