Immunohistochemical localization of CB1 receptor in canine salivary glands

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Abstract CB1 is a member of the G-protein-linked receptor superfamily that is present in the central nervous system as well as in certain peripheral neuronal and non-neuronal tissues. Recently, the presence of CB1 was found in the ductal system of the major salivary glands of laboratory animals, but no data are available for domestic mammals. Thus, in the present study, we examined the presence and distribution of CB1 in the major salivary glands of dogs using immunohistochemical techniques. CB1 was found in the parotid and mandibular glands of adult dogs; positive immunoreaction was localized to the cells of the striated ducts, with a peculiar localization on or near the apical membrane. This particular localization may be explained by the characteristics of this receptor as membrane-associated. The acinar structures were completely negative for CB1. We conclude that CB1 is involved in the control of dog salivary secretion via endogenous substances, likely endocannabinoids. The localization of CB1 highlights that endocannabinoids promote qualitative and/or quantitative changes of the primary saliva in the ductal system.

Keywords Cannabinoid receptors · Immunohistochemistry · Salivary glands · Dog

Abbreviations

CB1 cannabinoid receptor type 1
CB2 cannabinoid receptor type 2
ABC avidin-biotin complex
DAB diaminobenzidine

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Introduction

Cannabinoids are natural components derived from the plant *Cannabis sativa* whose pharmacological properties have been known for many years. Recently, interest in these substances has increased as a consequence of the cloning of two receptors specific to cannabinoids, cannabinoid receptor type 1 (CB1) and CB2, in humans (Munro et al. 1993) and laboratory animals (Matsuda et al. 1990), and the discovery of endogen substances, commonly indicated as endocannabinoids (Busch et al. 2006), that bind to these receptors.

CB1 receptors are members of the superfamily of G-protein-linked receptors. They are expressed in the central nervous system (Wenger et al. 1999; Pagotto et al. 2001), as well as in several neuronal and non-neuronal peripheral tissues (Osei-Hyiaman et al. 2005; Cavauto et al. 2007). Recently, the presence of the two receptors has been demonstrated in the major salivary glands of rats by means of immunohistochemical techniques (Busch et al. 2004; Prestifilippo et al. 2006). In particular, the CB1 receptor has been localized exclusively in the ductal system, while the CB2 receptor in the acini. These results allowed the “cannabinoid system” to be associated with two fundamental steps of salivary production: secretion of primary saliva from glandular acini and its modifications in the ductal system (Prestifilippo et al. 2006).

Currently, there is no literature examining the distribution of the two receptors in the salivary glands of domestic animals. Therefore, the purpose of this work was to characterize the cell type localization and distribution of the CB1 receptor in the major salivary glands of dogs by means of immunohistochemical techniques.

Materials and methods

Parotid and mandibular glands were obtained during autopsies of adult dogs without apparent lesions to the major salivary glands. The specimens were immediately fixed by immersion in 4% formalin in PBS for 24 h at room temperature, dehydrated, and embedded in paraffin. Five-micrometer thick serial sections were collected on poly-L-lysine-coated glass slides and processed for the immunohistochemical reaction following antigen retrieval with a microwave oven using 0.01 M citrate buffer, pH 6.0 (three cycles, each lasting 5 min). All subsequent steps were carried out in a moist chamber at room temperature. To prevent non-specific binding of primary antibodies, the sections were pre-incubated for 30 min with normal horse serum (1:10, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Subsequently, sections were incubated overnight with the anti-CB1 primary polyclonal antibody (1:100; sc-17555, Chemicon International Inc., Temecula, CA, USA). The next day sections were washed in PBS and incubated for 30 min with biotinylated secondary antibody, a horse anti-goat IgG (1:200; Vector Laboratories, Burlingame, CA, USA). The antigen-antibody complex was visualized using an avidin-biotin system (ABC, Vector Elite Kit, Vector Laboratories) for 30 min. The reaction was developed using diaminobenzidine (DAB, Vector Laboratories) as a chromogen. Positive and negative controls were included in the study.

Results

This immunohistochemical study revealed a strong immunoreactivity for the CB1 receptor (Figs. 1 and 2, arrows) in the cytoplasm of the striated duct cells of the salivary glands in all the animals studied. Such immunoreactivity was prevalently localized near the apical membrane of

Fig. 1 CB1 immunohistochemistry reaction in the dog parotid gland. CB1-staining was prevalently localized near the apical membrane of the striated ductal cells (arrows), while the acinar structures were negative (asterisks)

the ductal cells. The remaining cytoplasm of the same cells appeared completely negative. Likewise, the acinar structures were also negative (Figs. 1 and 2, asterisks).

Discussion

This study confirms the presence of the CB1 receptor in the major salivary glands of dogs and that CB1 localizes to the cells of striated ducts, as previously demonstrated in rats (Prestifilippo et al. 2006; Busch et al. 2006). The immunohistochemical positive reaction

Fig. 2 CB1 immunohistochemistry reaction in the dog mandibular gland. The reaction showed the same pattern of distribution as in the parotid with CB1 localized in the striated ductal cells near the apical membrane (arrows), while the acinar structures were negative (asterisks)

inside the cells was localized on or near to the apical membrane. This finding reflects the characteristics of this receptor that it belongs to the superfamily of G-protein-linked membrane receptors. No reactivity was observed in the acinous cells, which correlated to the immunohistochemical results in the rat studies (Prestifilippo et al. 2006).

Due to the presence of CB1 receptors in canine major salivary glands, we hypothesize that endogenous substances, attributable to the “endocannabinoids,” could attend salivary production in dogs. In particular, through the CB1 receptor, endocannabinoids could intervene in the saliva produced by acinar structures, causing qualitative and quantitative modifications of the primary saliva in the ductal system. Moreover, the aforementioned data seem to be in agreement with the observation that, in humans, the use of marijuana causes a reduction in the amount of saliva produced. Nevertheless, further studies are necessary to examine the possible coexistence of the CB2 receptor in the same glands and to understand more completely the biological meaning of the “cannabinoid system” in dog salivary glands.

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