FOLIA HISTOCHEMICA
ET CYTOBIOLOGICA
Vol. 48, No. 2, 2010
pp. 306-310

Immunolocalization of PTHrP in the parotid glands of three rodents species: Clethrionomys glareoulus, Microtus arvalis and white Swiss mice

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Abstract: The current study was inspired by the fact that since 2004 no report had appeared on the occurrence of this peptide in healthy parotid glands of humans and animals. The objective of the current study was to investigate the immunolocalization of PTHrP in the parotid gland of three male rodents: 6 common voles (*Microtus arvalis*, Pallas, 1779), 6 bank voles (*Clethrionomys glareoulus*, Schreber, 1780) and 6 white Swiss mice, as well as to find out any species differences in the distribution of this peptide in various types of cells of the parotid gland. Immunocytochemical reactions were performed using the ABC technique with specific rabbit antibodies against human PTHrP (34-53) (CALBIOCHEM), diluted 1:70 and 1:50. We observed positive PTHrP expression in the epithelial cells of the striated duct in all the three animal species. The expression was strong in white mouse and very strong in common vole and bank vole. In all the rodent species studied, the reaction for PTHrP was granular in nature and irregularly distributed in the cytoplasm, being definitely stronger at the base and weaker at the apex of the cells. The PTHrP expression was negative in the epithelium of the intercalated duct, interlobular duct, main excretory duct, as well as in the myoepithelial cells surrounding the excretory ducts or serous acini.

Key words: PTHrP, immunocytochemistry, parotid glands, rodents

Introduction

Parathyroid hormone related peptide (PTHrP) was identified in 1987 while investigating humoral hypercalcemia (HHM) in patients with neoplastic disease. In the course of neoplastic disease, PTHrP causes hypercalcemia, hypophosphatemia and elevated cAMP values in urine [1-3]. The human *PTHrP* gene occurs on the short arm of chromosome 12 and consists of nine exons [4]. Two of these exons are found unchanged in PTHrP transcripts. The remaining seven exons are localized in altered combinations in PTHrP mRNA, which allows the formation of approximately 15 various transcripts [1]. The expression of *PTHrP* gene is regulated mainly on the transcription level through IL-2, TGF-β, IGF-I, EGF. The PTHrP mRNA is responsible for the formation of three peptide isoforms with various chain length: 139, 141 and 173 amino acids [4-7]. Human tissues and neoplastic tumors may contain three transcripts, but some tissues show preference for one of the isoforms [4,5,8].

Later studies have shown that PTHrP is produced not only in neoplastic tissues but also by many healthy human and animal tissues: thyroid gland, parathyroid gland, pituitary gland, salivary gland, urinary bladder, kidney, mammary gland, testis, epididymis, cartilage, osteoblasts, vascular smooth muscle cells [2,8-26]. PTHrP is a very conservative peptide. Its presence has been detected in the preimplantation period.

PTHrP expression has been identified in the submandibular and sublingual glands of rodents (rat, white mouse, bank vole, common vole, pine vole) and in the human parotid glands, both healthy and those affected by inflammation or neoplasm [9,27-30].

The current study was inspired by the fact that since 2004 no report had appeared on the occurrence of this peptide in healthy parotid glands of humans and animals. The aim of the current study was to investigate PTHrP immunolocalization in the parotid gland of three rodent species (white mouse, common vole and

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Folia Histochem Cytobiol. 2010:48(2): 306 (306-310)
10.2478/v10042-010-0003-5

bank vole) as well as to assess any species differences in the distribution of this peptide in various types of parotid gland cells.

Materials and methods

Tissue collection. In the present study, we investigated parotid glands of adult male rodents: 6 common voles (*Microtus arvalis*, Pallas, 1779), 6 bank voles (*Clethrionomys glareoulus*, Schreber, 1780) and 6 white Swiss mice, which were obtained from the animal quarters of Białystok University. The mammals were anesthetized with 200 mg/kg pentobarbital sodium by intraperitoneal injection prior to cervical dislocation. The parotid glands were collected, fixed in Bouin's solution for 24 h and embedded in paraffin. Five µm sections were stained with hematoxylin and eosin (H+E).

Immunocytochemical procedures. Immunocytochemical reactions were performed using the ABC technique according to Hsu *et al* [31]. After blocking endogenous peroxidase activity with 1% H_2O_2 in distilled water, the sections were incubated for 30 min with normal goat serum (1:20). Next, they were incubated overnight at 4°C with specific rabbit antibodies against human PTHrP (34-53) (Calbiochem, USA), diluted 1:70 and 1:50. This was followed by 30 min incubation with biotinylated anti-rabbit antibodies and by 1 hour incubation with the streptavidin-biotinylated peroxidase complex. Peroxidase activity was detected with DAB. Between the successive steps, sections were washed in PBS (3 × 3 min). In negative controls, we omitted the primary antibody and used normal rabbit serum, the remaining stages being unchanged.

Results

We observed positive PTHrP expression in the epithelial cells that lined the striated duct in all the three animal species studied. Its intensity varied in a species-dependent manner (Figs. 1A, 1B, 1C). The expression was found to be high in white mouse (Figs. 1A), and very high in common vole and bank vole (Figs. 1B, 1C). In white mouse, some of the epithelial cells lining the striated duct showed a strong reaction, others exhibited a weak reaction, whereas in single cells a trace reaction was observed (Fig. 1A). The reaction was granular in nature and unevenly distributed within the cytoplasm, being by far stronger at the cell base and weaker at the cell apex (Fig. 1A). Both in bank vole and in common vole the epithelial cells of the striated duct showed strong PTHrP staining as compared to white mouse (Figs. 1A, 1B, 1C). In these two species (bank vole and common vole), the intensity of PTHrP expression in the epithelial cells of the striated duct was equally high (Figs. 1B, 1C). It was, however, much more varied in bank vole (Fig. 1B): in some cells the reaction was very strong, in others strong or weak and in some even negative (Fig. 1B). In common vole, the intensity of PTHrP expression in the epithelial cells of the striated duct ranged from very strong to strong and weak (Fig. 1C). The cytoplasm of the striated duct epithelial cells in common vole and bank vole showed irregular PTHrP staining (Figs. 1B, 1C). The reaction, granular in nature, was much stronger at the cell base as compared to the apical part, like in white mouse (Figs. 1A, 1B, 1C). The granular nature of PTHrP expression was still predominant (Figs. 1B, 1C).

In our study, PTHrP expressions in the epithelium of the intercalated duct, interlobular duct and main excretory duct were negative in all the three animal species studied. We found no PTHrP expression in the myoepithelial cells surrounding the excretory ducts or serous acini.

Discussion

We found PTHrP expression in the epithelial cells of the striated duct of the parotid glands in all the three animal species examined, but not in the epithelial lining of the intercalated, interlobular and main excretory duct. Our results are similar to those reported by other authors. Also Zabel *et al*. [30] did not observe the presence of PTHrP in the epithelium of the intercalated duct in the healthy human parotid gland. However, these authors, unlike us, detected positive PTHrP staining in the epithelial cells of the interlobular and main excretory duct in the healthy human parotid gland. Also Seidel *et al*. [27] showed the expression of PTHrP in all the epithelial cells lining the excretory duct, both intra- and interlobular duct, in the human parotid and submandibular salivary glands. Likewise, Sunardi *et al*. [28] described a positive reaction for PTHrP in the epithelial cells of all the segments of the excretory ducts in the healthy human parotid gland. On the other hand, Czykier *et al*. [26] observed PTHrP expression in the epithelial lining in all types of the excretory ducts in the submandibular glands of white mouse, bank vole and pine vole. In the sublingual glands of white mouse, bank vole and common vole, these authors [32] found the presence of PTHrP in the epithelial cells of all the excretory ducts, except for the intercalated duct. Our previous and current findings demonstrate a substantial organ variation in PTHrP localization in rodent salivary glands. At the same time, literature data and our results seem to indicate that PTHrP belongs to the peptides that frequently occur in the epithelial cells of the excretory ducts of the salivary gland in various animal species.

We believe that PTHrP found in our study in the epithelial cells of the striated duct of the parotid gland, just like in the renal tubules, can play a role in the transport of electrolytes, especially calcium ions, as this peptide, together with PTH, regulates calcium-phosphate balance in the body. This has been confirmed by the findings reported by Dua *et al*. [33] on the sheep salivary gland. These authors [33] observed after PTHrP infusion (1-34) a significant increase in the total and ionized calcium concentration in plasma but not in saliva. In contrast, they noted a significant

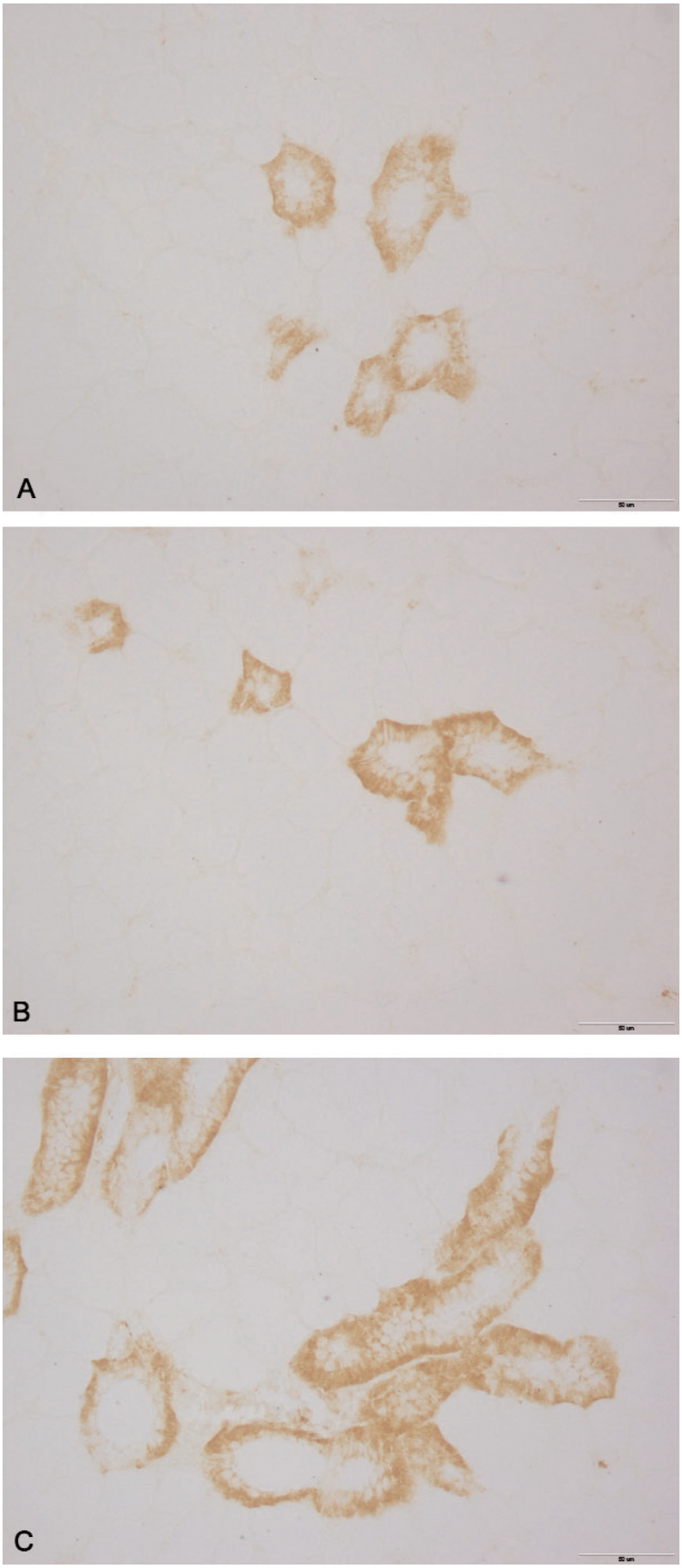

Fig. 1 PTHrP expression in the epithelium of the striated duct of parotid glands in **(A)** white mouse, **(B)** common vole, **(C)** bank vole.

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10.2478/v10042-010-0003-5

decrease in the plasma concentration of phosphate and potassium, and a corresponding increase in their salivary concentrations and clearance rates. Moreover, according to Esbrit *et al*. [34], PTHrP present in the epithelium of the renal canaliculi regulates their proliferation. In our opinion, PTHrP may have a similar role in the epithelial cells of the excretory ducts of the salivary glands in white mouse, bank vole and common vole.

Other authors have described PTHrP expression in the myoepithelial cells of the submandibular gland, mammary gland, small sweat gland and inflammatory human parotid secretory elements, in the culture of myoepithelial cells of the mammary gland [9,22,30]. Seitz *et al*. [35] have found out that PTHrP (1-34) causes an increase in intracellular cAMP in myoepithelial cell line derived from normal human breast. At the same time, these authors believe that PTHrP changes the intracellular Ca^{2+} response to oxytocin. We did not observe PTHrP expression in myoepithelial cells surrounding serous acini of the parotid gland.

Our findings concerning PTHrP expression in myoepitelial cells surrounding the excretory duct are consistent with observations reported by other authors, who have not detected PTHrP in these cells in any of the animal species examined [9,27,28,30]. Our current study concerning immunolocalization of PTHrP in the parotid glands of three rodents species is a continuation of our investigations of the occurrence of this peptide in submandibular and sublingual glands, and at the same time extends the knowledge of the subject that has not been researched since 2004.

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Submitted: 10 October, 2009
Accepted after reviews: 11 February, 2010