FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 51, No. 1, 2013
pp. 55–58

ORIGINAL STUDY

VIA MEDICA

Atrial Natriuretic Peptide presence in parotid gland of human fetus at 13th week of development and in adult man

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Abstract: Our immunohistochemical study shows that atrial natriuretic peptide (ANP) is present in both human fetal and adult parotid gland. In the fetuses ANP is localized in cell clusters, while in adult parotid gland, the ANP is seen only in the wall of intra- and extralobular ducts. The results suggest that ANP might play a role in the differentiation of the parotid gland similarly as in brain and that during fetal growth ANP may play a role in the regulating the secretion of primitive salivary fluid, even if acini are still not developed. *(Folia Histochemica et Cytobiologica 2013, Vol. 51, No. 1, 55–58)*

Key words: ANP, human parotid gland, ontogeny

Introduction

Atrial natriuretic peptide (ANP) is a polypeptide hormone mainly synthesized and excreted by cardiomyocytes. However, ANP is not only a cardiac hormone since it plays a role in the functions of many different organs such as brain [1, 2], kidney [3], turbinates [4], lung [5], pleura [6] and also pancreas [7]. Furthermore, since ANP plays an important role in the regulation of fluid and electrolyte homeostasis, this peptide may be also involved in the function of salivary glands.

Although in the literature there are few studies regarding the occurrence of ANP in the salivary glands of some rodents, particularly in rat, the results are uncertain. Cantin et al. [8] found that in rat parotid gland ANP immunoreactivity was intense exclusively in the acinar cells; in sublingual gland only the serous cells were immunoreactive, whereas in submaxillary gland the reaction was weaker and distributed randomly in the gland. By contrast, Gutkowska and Nemer [9] found the ANF-immunoreactivity confined to the granular convoluted tubular cells of the submaxillary gland. Furthermore, Vollmar et al. [10] found immunoreactivity in ductal cells in rat parotid gland. Recently Cho et al. [11] have showed in rat submaxillary gland a co-localization of C-type natriuretic peptide (CNP) and ANP in ducts and endothelial cells, however, not in acini. In rabbit, ANP was found in intralobular and extralobular ducts of the parotid gland [12].

The literature data on the ANP-ontogeny refer to heart [13–15], brain [16–18], and lung [19]. The morphology of developing salivary glands was described in rat [20, 21], mouse [22], miniature pig [23], and in humans [24]. Few reports referred to the presence of peptides in the developing parotid gland. Sivakumar et al [25] found that in rat parotid gland the amylase and parotid secretory protein (PSP) gene expression appeared early in development in the immature acinar cells (within the last few prenatal and first few postnatal days) and was maintained in acinar cells, but not in intercalated ducts of the adult gland; common salivary protein-1 (CSP-1) was expressed at relatively high level in the immature parotid gland acinar cells and in the adult gland it was specifically lo-

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calized to the intercalated ducts [25]. Ogawa et al. [26] found that in rat parotid gland keratin 14 (k14) appeared in myoepithelial cells, basal cells and striated ducts at 3 days after birth. Guanylin appeared in rat parotid gland anlage as a simple tree of solid buds and at the stage E17 no immunoreactivity was found within the gland [27].

Recently, in the human parotid gland Fanni et al. [28] found a strong expression of thymosin beta 10 (Tβ10) that during the initial phases of the development was localized extracellularly, at the 13th week of gestation in the cytoplasm of immature duct cells, at 20th week of gestation in acinar cells and in the duct lumen at 33th week of gestation. The presence of peptides during the development and their disappearance in the adult age suggest their role in the salivary gland organogenesis.

Although the ANP presence was studied in salivary glands of some rodent species, no study was reported regarding human parotid gland. Since many peptides are present in salivary glands during the development and disappear in the adult, we performed an immunohistochemical study in human parotid gland in fetus, at 13th week, and in the adult gland to establish if ANP was present in both fetal and adult life.

Material and methods

Four human fetuses of a gestational age of 13th week, obtained by natural aborts from the Obstetric and Gynecologic Clinic of University of Palermo, were fixed in Bouin's fluid; the whole head of each fetus was removed, fixed in the same fluid for 12 h, and afterwards dehydrated and embedded in paraffin. The parotid gland was easily identified in the sections because of its position in relation to other structure. Some sections were stained using hematoxylin-eosin method and some slides were immunostained.

Specimens of ten adult human parotid gland were collected by biopsies found normal by histolopathologist. The samples were fixed in Bouin's fluid, dehydrated in graded alcohols and embedded in paraffin. Seven mm thick sections were cut with Leica microtome RM2145, dried overnight at 37°C and then stored at room temperature until use. All samples were obtained with the consent of the local Ethical Committee.

Immunohistochemistry (IHC)

The slides were dewaxed in xylene and rehydrated in a graded series of alcohols and were then transferred into distilled water for 5 min. IHC was performed using the 'DakoCytomation EnVision + System-HRP (AEC) kit' from Dako (Dako, Glostrup, Denmark), following the manufacturer's instructions. Briefly, sections were covered with the 'Peroxidase block' reagent and incubated for 5 min. at room temperature. The samples were rinsed once in PBS buffer pH 7.2. The sections were covered with rabbit anti-ANP polyclonal antibody (Chemicon, Temecula, CA, USA) and incubated at 4°C overnight. The antibody was diluted in a 0.1% BSA solution at the dilution of 1:800. Samples were rinsed twice in PBS pH 7.2 and then incubated with the 'Peroxidase Labelled Polymer' reagent for 30 min. Samples were rinsed twice in PBS pH 7.2, and then incubated with the 'Substrate-Chromogen' reagent and immediately observed under a light microscope; the reaction continued until staining appeared (2–10 min.). Reaction was stopped by rinsing the slides in distilled water. Negative control sample was treated in the same way, however, without the use of primary antibody. Slides were coverslipped using the 'Dako Cytomation Faramount Aqueous Mounting Medium' from Dako (Dako, Glostrup, Denmark). The specimens were observed under a Leica DM1000 light microscope.

Results

In the human fetus at 13th week of gestation, parotid glands showed a considerable development; a connective tissue outlined the gland; in the abundant mesenchyma many scattered cell clusters without a cavity were present and, also, some elongated primitive excretory tubular structures with epithelium with the prismatic cells limiting a cavity occurred, however, the acini were not recognizable (Figure 1A).

In the ANP-immunostained sections, ANP presence was noted in the apical area and also in the cytoplasm of numerous cells of cell clusters and tubules; however, many cell groups were immunonegative. (Figures 1B and C).

In the adult gland, the acini were composed of serous cells limiting the cavities that continued with intralobular ducts leading to interlobular ducts. The ANP-immunostained sections showed acini with immunonegative serous cells, the intralobular ducts with epithelial immunopositive cells (Figure 1D) and the extralobular ducts with ANP-immunopositive epithelium (Figures 1D and E). Particularly, in the ductal epithelium the basal cells were immunopositive and also the columnar cells presented ANP-immunopositivity in the basal and perinuclear area (Figures 1E and F).

Discussion

The ANP presence in the salivary glands in different species of animals and in humans suggests that ANP may play an important role in these organs, because

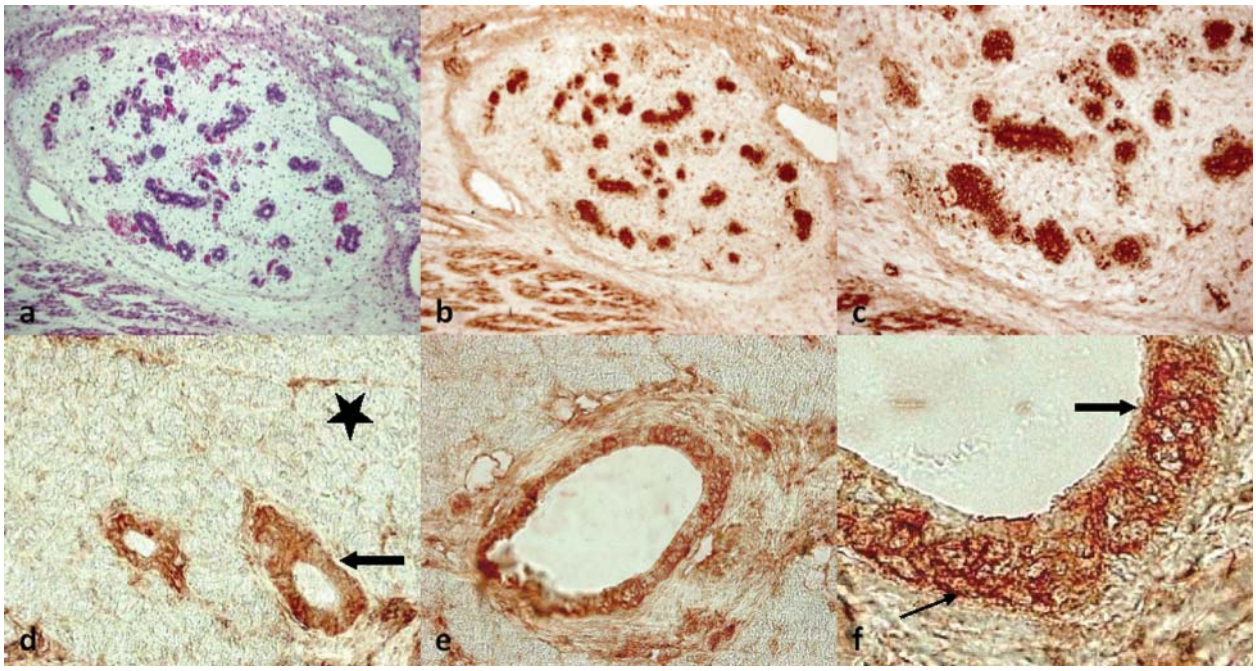

Figure 1A. Parotid gland of human fetus at 13th gestation week. Connective tissue capsule is surrounding the gland. Cell clusters and tubules are present in mesenchyma. Hematoxylin-eosin. Original Magnification 10 ×; **B.** Parotid gland of human fetus at 13th gestation week. ANP immunopositive cell clusters and tubules in the mesenchyma 10 ×; **C.** ANP-immunopositive cell clusters and tubules in human fetus parotid gland 20 ×; **D.** Adult human parotid gland: ANP-immunonegative alveoli (star); ANP-immunopositive intralobular ducts (arrow) 20 ×; **E.** Adult human parotid gland: ANP-immunopositive interlobular ducts 20 ×; **F.** Adult human parotid gland: ANP-immunopositive columnar (thin arrow) and basal cell (thick arrow) in the ductal epithelium 40 ×

this peptide is involved in regulation of the composition and concentration of electrolytes in the saliva [29], for example ANP enhances the salivary secretion induced by pharmacological agents in rat [30]. The ANP presence in parotid gland of adult rat [8, 10] and rabbit's pleura [10] was demonstrated.

Our study on human parotid glands of fetuses and adult specimens showed that in the human fetus, at 13th week of development, the architecture of the parotid gland was still undefined, since the acini were absent, and only cell clusters and ducts were indistinctly or indistinctly recognizable. Due to the IHC staining ANP presented a strong immunopositivity in cell clusters and elongated cell formations. The adult human parotid gland showed the known architecture of the gland with the acini composed of an unique cytotype, serous cells, and excretory intra- and extralobular ducts. Staining by the IHC method showed that the acinar cells were ANP-immunonegative, while the cells of the excretory ducts were intensely ANP-immunopositive; particularly, the ductal cells presented ANP-immunopositivity close to the apical area of cylindrical cells and also in perinuclear area of basal cells. The ANP presence in the ducts was probably related to the natriuretic activity of ANP, since saliva is an ion-rich fluid which content becomes modified during passage along the ductal epithelium in both intra- and extralobular ducts, where most of the NaCl is being reabsorbed [31]. Furthermore ANP in human parotid gland has the same localization as in rat [8, 10] and rabbit [12].

The comparison of human fetal and adult parotid glands evidenced that the ANP was present in both fetal and adult parotid gland. Since in the adult gland the acini were immunonegative and the ducts were immunopositive, it can be assumed that in the 13-week old fetus all the ANP-immunopositive cell clusters may be considered as cells of the primitive ducts with still undifferentiated acini. Thus, we suggest that the appearance of the ANP might be a marker to identify and to establish the time of the formation of the acinar cells. The ANP presence in the intralobular and extralobular ducts suggests that in the parotid gland's excretory ducts ANP plays a role in the regulation of the electrolytes concentration in the secretive fluid by its action affecting sodium-concentration and body water volume in both human fetal and adult body. Moreover, ANP presence in fetus indicates that the parotid gland acquires functional activity at early developmental stage.

Many molecules are involved in the differentiation of tissues and organs. For example, cholinesterases in mesenchymal cells play an inducing role in the morphogenesis of lungs [32] and somatostatin affects brain development in rat [33]. In regard to the salivary glands, Fanni et al. [28] indicated that thymosin â10 has a role in their organogenesis. Based on the established data about ANP role in rat brain development [15, 16, 22], our results showing ANP presence early during the development of human parotid gland suggest that ANP might be involved in parotid gland's maturation similarly as in the nervous system.

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Submitted: 19 July, 2012
Accepted after reviews: 8 February, 2013