Intraspecific Differences Regarding Granular Polymorphism in Granular Ducts' Cells in Rats' Mandibular Gland

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Abstract

Mandibular gland ducts' system in rodents consist of intralobular ducts (intercalated, granular, striated) and interlobular one (main excretory duct). Granular ducts are located between intercalated and striated ducts, being present only in mandibular gland of the mouse, rat, hamster and gerbil. The biological material used for this study was represented by two strains from the same species, three Wistar rats and three Brown Norway rats. After the animals were euthanized, the mandibular glands were harvested and then processed for histological investigations. The tissue fragments were sectioned at 5µm thickness and then stained the sections using Tricrom-Goldners method. Our results emphasize that the granular ducts are well developed; regarding the shape, they are convoluted in both Wistar and Brown Norway rats, without any significant differences between the two strains. In Wistar rat, the granules in granular ducts cells are small to medium in size, with discrete polymorphism. In Brown Norway rat, the cytoplasm is loaded with granules as in Wistar rat, but these are several times larger and more polymorphic.

Keywords: granular ducts, intraspecific, mandibular gland, rat.

Introduction

In rodents, major salivary glands consist of three pairs: parotid, mandibular and sublingual. Through excretory ducts, these glands are secreting a serous, mucous or mixed secretion. Regarding salivary ducts, a series of discoveries were made in the seventeenth century by Niels Stensen (1638-1686), Thomas Wharton (1614-1673) and Caspar Bartholin (1655-1738). Therefore, they established the concept of exocrine secretion (Lydiatt and Bucher, 2012).

The saliva is secreted into the oral cavity through the ducts' system, having various functions (lubrication, antibacterial, buffer system, digestive etc.). Clinicians and pathologists study the anatomy and histology of human tissues, being interested in normal morphology and function, using laboratory animals as experimental models. Due to bioethical and technical limitations on human tissue manipulation as well as advances in laboratory animals, the need for rodent tissue research has increased. Thus, more information is needed about the salivary glands of humans and, implicitly, rodents (Bazan *et al*., 2001).

Rodents' mandibular duct system consists of intercalated, granular, striated and interlobular ducts. Intercalated ducts are located between acini and granular ducts, receiving primary saliva directly from the acinus. Granular ducts are present only in mandibular gland of mouse, rat, hamster and gerbil (Pinkstaff, 1980; Mori *et al*., 1992; Bazan *et al*., 2001) and are located between intercalated and striated ones. Striated ducts are part of the duct system specialized for secretion and

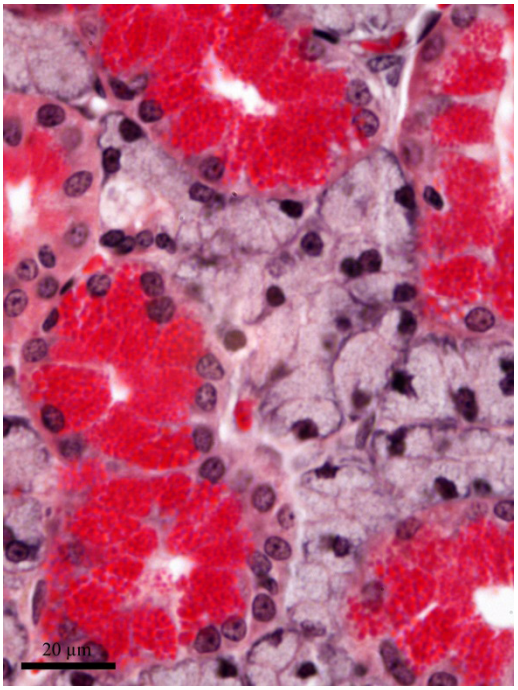

Figure 1. Portions of granular ducts composed of tall cells, with the cytoplasm filled with acidophilic secretory granules in mandibular gland in Wistar rat strain (Tricrom-Goldner stain)

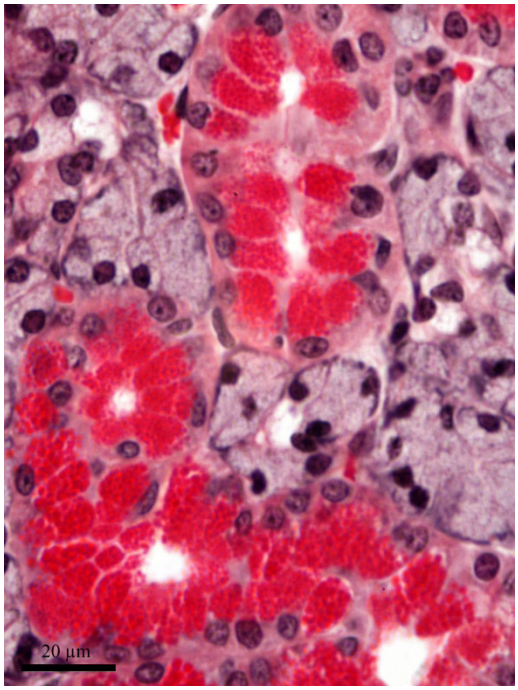

Figure 2. Secretory granules in Wistar rat strain (Tricrom-Goldner stain)

absorption of electrolytes, transported between the duct lumen and extracellular spaces (Amano *et al*., 2012). There are two types of secretory granules in mandibular gland of adult rat: one type is found in acinar cells and the other in granular ducts. The acinar cell granules are mucosal and those of granular ducts are serous (Bivin *et al*., 2013). Considering that we did not found any concrete information about the existence of some possible differences between the secretory granules of the granular ducts of the mandibular gland in rodents, we wanted to see if there are any histological differences between the two strains of rats raised in identical life conditions.

Materials and methods

The biological material for this study was represented by males of two different strains of the same species: three Wistar rats and three Brown Norway rats, 13 weeks old, from University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca Biobase. The investigation was approved by the UASVM Bioethics Committee of Cluj-Napoca and was carried out in accordance with the Ministry of Health legislation. Following euthanasia by prolonged exposure to Isoflurane inhalation anesthesia, the mandibular glands were harvested and processed for histological investigations. The samples were fixed in 10% formalin, dehydrated in alcohol with increasing concentration (70°, 95°, absolute), clarified with n-butanol and included in paraffin. Samples of 5 µm thickness were stained with Tricrom-Goldner method. The samples were examined at an Olympus BX41 microscope equipped with a digital camera for capturing images.

Results and discussions

Histologically, mandibular gland' structure shows comparable structure in both strains of rats. Granular ducts are well developed; regarding the shape they are convoluted in both Wistar and Brown Norway rats, without any significant differences between these two strains. Granular ducts' cells have similar height and acidophilic granules that occupy more than half of the cytoplasm in both Wistar and Brown Norway rats.

In addition to the aspects previously described, there are also differences between granular ducts cells in the two rat strains. In Wistar rat, the granules are small to medium size, with discreet polymorphism in most granular ducts (Fig. 1; Fig.

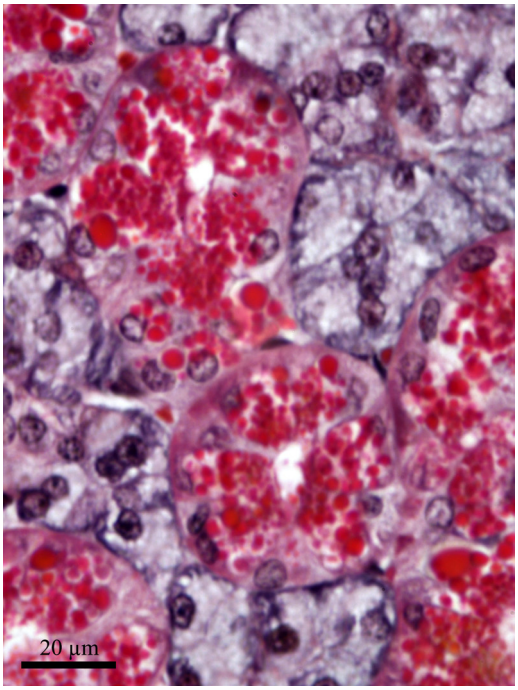

Figure 3. Polymorphism of the secretory granules in Brown Norway rat strain (Tricrom-Goldner stain)

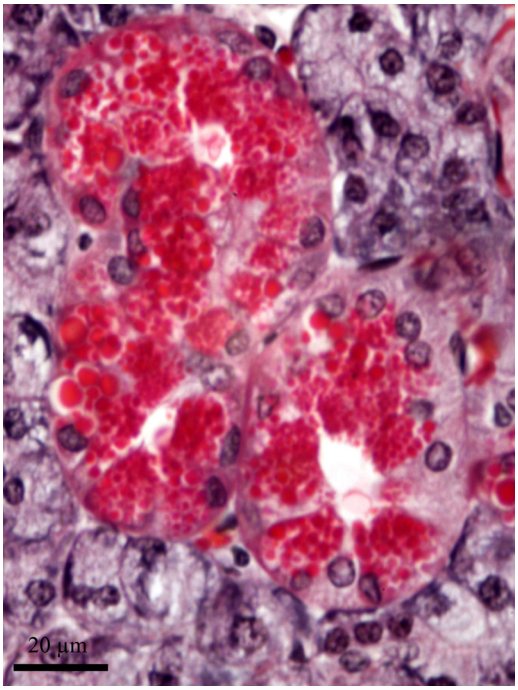

Figure 4. Portions of granular ducts composed of tall cells, with the cytoplasm filled with acidophilic secretory granules in mandibular gland in Brown Norway rat strain (Tricrom-Goldner stain)

2). There are also some larger granules but they are present only in a small number of cells and only in some of the ducts. In Brown Norway rat, the cytoplasm is equally loaded with granules, but they are different in size and polymorphism compare with Wistar rat (Fig. 3). Even though the shape of the granules is more or less spherical as in Wistar rat, the size of the granules in granular ducts cells in Brown Norway rat is significantly larger and the polymorphism are more pronounced (Fig. 4). The majority of the granules are several times higher in Brown Norway rat compared to Wistar rat and above that, there is a significantly larger number of large and very large granules. Such granules appear from the fusion of other smaller granules (granules' aggregate). In Brown Norway rat not only the number of these very large granules is superior to those in Wistar rat, but also two or more such aggregates are frequently present in the cytoplasm of the same cell (Fig. 4). There is a certain number of cells in which such large aggregates occupy most of the cytoplasm. Between these aggregates there are smaller or larger differences in diameter, meaning that they are also very polymorphic.

The presence, shape, size and location of the granules in cytoplasm of granular ducts cells in rodents' mandibular gland have been the subject of numerous studies.

Some authors assert that the development of granular ducts takes place at the terminal portion of the striated ducts. They claim that two weeks postnatal, in these cells appear the first granules (10-20 at the beginning), small in size (0.2-0.4 µm). The number and size of intracytoplasmic granules grow directly proportional to the age of the animals. Along with this, there are increasing variations of the size of the granules (0.2-1.5 µm) (Cutler and Chaudhry, 1975; Srinivasan, 1975).

Cutler and Chaudhry (1975) claim that at eight weeks, after the accumulation of apical granules, the granular ducts become mature and practically finish the process of transformation from the striated ducts into granular ones. The size of the granules may increase so that at 14 weeks, most of the granular ducts cells have relatively large apical granules (1.12-1.75 µm).

The granules in granular ducts cells cytoplasm have the same electronic density and generally have round shape but they have not the same size (Mori *et al*., 1992). There are also authors who claim that the number of secretory granules in granular ducts increase in rainy season in males

(Cangussu, 2002). All authors agree with the location of granular ducts, being situated between intercalated and striated ducts (Igbokwe *et al*., 2015).

Most authors say that the granular ducts cells contain numerous granules in the apical cytoplasm (Igbokwe *et al*., 2015), occupying the upper half of the cell. The granules are round, delimited by a membrane and have dimensions from 0.2 to 2 μm in diameter. In each cell there are some very small and rarely very large granules (> 5μm in diameter). Measuring the diameter of the granules, Hazen-Martin and Simson (1986) (cited by Gresik, 1994) found that small granules confined from 0.2-0.4 μm and the large ones from 1.0-1.3 μm in diameter, with few granules of intermediate size. Some cells have relatively small size granules and occupy the apical third of the cell. It appears that such granules probably have cells that have recently eliminated secretion products and reconstruct their deposits of secretion materials (Hosoi *et al*., 1978; Gresik 1994).

Regarding the surface occupied by the granules in granular ducts' cells cytoplasm, we found slightly different results from those in scientific literature, meaning that in both rats strains, the granules occupy more than the apical half (60-75%). We also identified big differences between both rats strains in terms of granule size and polymorphism. We have not identified such data in the studied literature so it seems that this is the first mentioning of intraspecific differences regarding the size and polymorphism of the granules. We can not appreciate what functional significance these differences have on the basis of histological investigations. We cannot appreciate why in Brown Norway rat the process of granules merging with the result of larger granules or even conglomerates is obviously more intense than in Wistar rat. In addition, we cannot say whether this is an advantage, a disadvantage or has no functional significance. There may be differences between the two rat strains related either to the intensity of the secretion or the rate of elimination of the secretion from granular cells.

Conclusion

Our study reported for the first time in literature the existence of significant differences between granular ducts cells in two strains of rats, Wistar and Brown Norway, raised with identical life conditions. These differences refer to the size and polymorphism of intracytoplasmic granulations, which are significantly higher in Brown Norway rat compared to Wistar rat.

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