Holocrine secretory mechanism in granular ducts in Brown Norway rat. Histological study

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Abstract

Mandibular glands in rodents contain a particular type of ducts, namely granular ducts. The cells lining these ducts present granules in their cytoplasm and secrete different substances. Our study aimed to assess these granules and the secretory mechanisms of these cells. The biological material was represented by 5 adult males Brown Wistar rats. We harvested the mandibular glands and processed them for histological examination. The slides showed that the cells lining the granular ducts present granules of different sizes with a spherical shape. They can occupy up to half of the cell cytoplasm and sometimes even more, forming large aggregates. The secretion of these large aggregates takes place through a holocrine secretory mechanism. The cells presenting this type of mechanism can be easily identified on the slides because they show discontinuity of the apical pole and a tendency of dispersion of the cellular contents. The other two types of secretory mechanisms are also present. In other words, the cells lining the granular ducts from Brown Norway rats mandibular glands, present merocrine, apocrine and holocrine secretory mechanisms. This is the first evidence of the holocrine mechanism cells from granular ducts in mandibular gland in this species.

Keywords*: granules, holocrine, mandibular, Brown Norway rat.*

Introduction

Granular ducts from rat mandibular glands are lined by cells containing obvious granules (Matthews, 1974a). Along time, researchers considered that these granules are not secretory, because they did not observe signs of their exteriorization nor abundant endoplasmic reticulum, which would suggest the fact that the cells are secretory. Recent publications mentioned that there are signs of exocytosis observed quite often, which confirment the secretory nature of the cells lining the granular ducts from rat mandibular gland (Giebisch, 2013).

In rodents (mouse, rat, hamster etc.), granular ducts from the mandibular gland are interposed between intercalary and striated ducts. They secrete proteases and bioactive polypeptides (different growth factors) (Mori et al., 1992; Tandler et al., 2001; Cha, 2017). These ducts are encountered in large numbers in males because they are androgen-dependent. Thus, the granular ducts present sexual dimorphism (Cha, 2017), being much more developed in males (Frith and Townsend, 1985; Amano et al., 2012). At birth though, the gland is immature and does not contain granular ducts, which form only later (somewhere in the interval 1-3 months of life) (Hecht et al., 2000, Coire et al. 2003).

Granular ducts are lined by more types of cells as follows: dark narrow, light granular and dark granular. The dark narrow cells contain a lot of free ribosomes (free-floating), but no endoplasmic reticulum or granules. The second cellular type, the light granular cells present a variable endoplasmic reticulum quantity and granules, while the third type is filled with granules, and the cellular organelles, the cytoplasm, and nucleus are pushed towards the basal zone. It seems that these cellular types would be secretory stages of the same cellular type, fact that would sustain

the affirmations according to which, the secretion of this cellular type is cyclic and not continuous (Tamarin and Sreebny, 1965).

Material and methods

The experimental study unreeled with the accord of the Bioethics Committee of the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca. The utilized animals were kept in the biobase of the Faculty of Veterinary Medicine in Cluj-Napoca and were represented by 5 Brown Norway rats.

Immediately after sacrification, the mandibular glands were harvested for histological investigations. The samples were immersed in 10% buffered formalin immediately after harvesting and maintained in the fixation solution at room temperature, for 5 days. The utilized formalin was prepared 5 days before using it, from 20 ml concentrated formalin and 180 ml distilled water. During the fixation period, we changed the fixation solution 3 times, so that the fixation would be appropriate. The proportion of the volume of the sample and the one of the fixation solution was 1:40 (Kiernan, 1990).

After the fixation period was finished, the samples were immersed in successive baths of alcohol, in increasing concentrations, as follows: 70^0, 95^0 and absolute. At the end of the dehydration period, the samples were clarified with n-butanol. The paraffin infiltration was achieved at a 56^0C temperature, after which the samples were immersed in melted paraffin and were left at the laboratory temperature to solidify. After shaping the paraffin blocks in which the sample was included, we obtained seriated sections of 5 µm thickness with the aid of a Leica rotary microtome. After mounting on histological slides, the contrasting technique used was Goldner's trichrome staining procedure.

The histological slides were examined under an Olympus BX41 light microscope and the photographs were taken with a photo camera (E-330), attached to the microscope. The subsequent processing of the photographs was performed with the aid of Adobe Photoshop CS2 software.

Results and discussions

The mandibular gland in Brown Norway rats resembles the one in albino Wistar rat and albino laboratory mouse from a histoarchitectural point of view, in the sense that it presents very well developed granular ducts. The cells lining these ducts are tall and have a cytoplasm filled with acidophilic granules with different sizes and spherical shape (Fig. 1).

What comes forward, is the presence of very large granules in some cells, occupying a major part of the cytoplasm (Fig. 2).

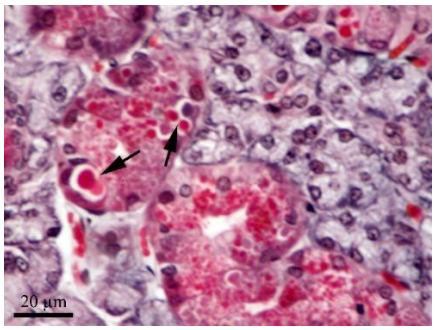

Fig. 1. Mandibular gland in Brown Norway rat – Acidophilic granules in the cells lining the granular ducts (black arrows)

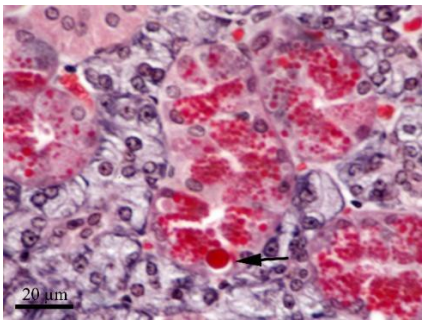

Fig. 2. Mandibular gland in Brown Norway rat – Large granules in the cells lining the granular ducts (black arrow)

These cells can present one or more such granules, which suggests that they form by fusion of smaller granules. The aspect was signaled by other authors who sustain that the granules from the cytoplasm of cells in granular ducts existent in some rodents can fuse in order to form larger granules or even aggregates of large and sometimes very large dimensions (Thomopoulos et al., 2002).

In mandibular gland from Brown Norway rats taken into study, we highlighted a remarkable polymorphism of the intracytoplasmatic granules, which suggests the fact that the granular aggregation process is not only present, but also very intense. Moreover, the evolution of granule fusion until large size conglomerates (aggregate) formation is frequent. One or more such aggregates can be found in the cytoplasm of one cell so that in some cases, they can occupy up to half of the cytoplasm or even more (Fig. 3). In most of the cases, the large conglomerates are accompanied by a certain intracellular oedema, materialized on the microscopical image through a clear halo, surrounding the structure (Fig. 4).

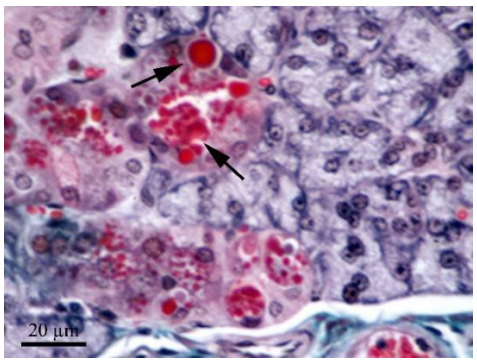

Fig. 3. Mandibular gland in Brown Norway rat – Aggregates in the cells lining the granular ducts (black arrows)

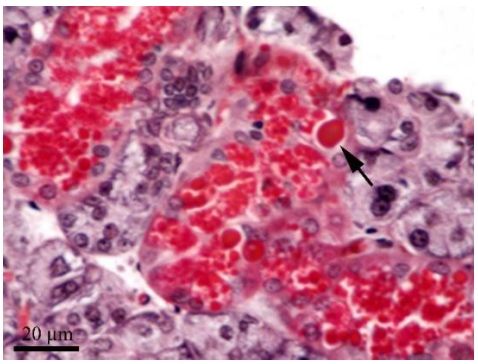

Fig. 4. Mandibular gland in Brown Norway rat – Clear halo around an aggregate (black arrows)

Needless to say that the presence of such structures in the cytoplasm of the cells disturbs the normal unreel of the cellular metabolism. Moreover, these cells have a secretory activity and the secretory products accumulated in the intracytoplasmatic secretion granules have to be eliminated from the cells when they are needed. Elimination of the secretory products from the small sized granules is possible through a merocrine secretory mechanism (reversed pinocytosis) (Amano et al., 2012). Also, these granules and even the larger ones (medium size) can accumulate in the apical pole of these cells in order to be eliminated through an apocrine secretory mechanism, also signaled before in the cells from granular ducts (Messelt, 1982; Messelt and Dahl, 1983). Some authors signal the formation of these aggregates even in the striated ducts of the mandibular gland in slow loris (*Nyctecebus coucang*) (Tandler et al., 1996; Tandler et al., 2006). The authors mention that filaments are present in the apical pole, which associate with the membrane surface and help the large granules move towards the surface in order to be exocitated (Tandler et al., 1996; Tandler et al., 2001). The question arises whether large conglomerates can be eliminated through one of the two secretory mechanisms (merocrine and apocrine) signaled in the scientific literature. We do not think such a thing is possible because the dimension of some granules is too large for the two secretory mechanisms to be functional. Moreover, their presence and persistence in the cytoplasm of the cells elicit an imbalance which will lead at some point to dysfunctionality of the cell, which will eventually disintegrate. By rupture of the cellular membrane, the conglomerate (or conglomerates) will be eliminated along with the other granules present in the cell. In this situation, these cells eliminate their secretory products through a holocrine secretory mechanism

(disintegration of the cell which produced them), aspects present on the sections we made in the mandibular gland in Brown Norway rat. The aspects we intercepted clearly suggest that besides the secretory mechanisms signaled in the scientific literature (merocrine and apocrine), in Brown Norway rat, the holocrine secretory mechanism is also present. We did not find any information in the scientific literature regarding the presence of holocrine secretory mechanism in the mandibular gland in Brown Norway rat. Given the situation, it seems like this is the first evidence of the presence of holocrine secretory mechanism in the mandibular gland, in Brown Norway rat. We have to mention that this mechanism is not necessarily predominant in the mandibular gland in Brown Norway rat, but is relatively well represented. It is present as mentioned before in cells in which large granulations or conglomerates are formed, but also in other cells which present small or at most large granulations. These cells are relatively easy to identify in a histological investigation because they present discontinuities (ruptures) of the apical pole and a tendency of dispersion of the cellular contents. Such phenomena can be observed in either isolated cells, larger or smaller groups of neighbouring cells, presenting clear signs of structural disintegration. Some are intercepted when only a part of the granules, regardless of the size, were eliminated, but others appear void of contents. The situation highly differs from one duct to another, which determines us to think that the secretion is not synchronized not only from one duct to another but also from one area to another of the same duct. This asynchronous secretion is mentioned by Tandler et al. (2001) in striated ducts, who state that it can be encountered in different species. Also, they mention the fact that the secretory granules differ a lot between the cells. It seems that the secretion rhythm and the mechanisms through which the secretory product is eliminated from the cells are adjusted to the functional necessities of the gland and maintained between physiological limits. If the histological investigation allows the assessment of the size and aspect of the granules in the cytoplasm of the cells lining the granular ducts in Brown Norway rat, it does not offer information on the phenomena determining the fusion of granules with the formation of large granules and conglomerates. In this situation, we are bound to only signal their presence, without being able to state if their formation is an advantage or disadvantage for the animal. It is possible that they do not have any functional meaning, especially if the substances they contain do not suffer structural changes. In such a situation, the substances will be liberated through the disintegration of conglomerates in the lumen of the excretory ducts, which transport the secretion products and subsequently released for the organism to use, same as the small sized granules.

The absorption of tritiated tryptophan in the cells lining the granular ducts was studied and a slow turnover process of the secretory proteins was observed (Matthews, 1974a). The sympathetic stimulation led to cell degranulation, while stimulation of parasympathetic nerves did not yield any response regarding the secretion (Matthews, 1974b). It seems that the granules form again 8 hours after degranulation.

The authors mention that the secretory granules seem to be serous because they are electrono-dense and contain glycoproteins in small quantity. The granules are polymorph, suggesting a fluid consistency. The membrane of two granules can fuse on a larger or smaller distance or they can fuse with the cell membrane, forming a pentalaminar membrane. Yet, the authors did not observe other signs of exocytosis (Matthews, 1974b; Giebisch, 2013).

Our study revealed that the cells lining the granular ducts in mandibular gland of Brown Norway rat release their secretory granules in three different ways. We found evidence of holocrine secretory mechanism, which was not mentioned in the scientific literature so far.

Conclusions

The cells lining the granular ducts in the mandibular gland in Brown Norway rat present the three types of secretory mechanisms, but only two of them are mentioned in the scientific

literature: merocrine and apocrine. Some cells present a holocrine secretory mechanism materialized through the disintegration of the cells, with the elimination of the cellular content. This is the first evidence of the holocrine secretory mechanism in cells from granular ducts in mandibular gland in Brown Norway rat.

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