COMPARATIVE STEREOLOGICAL STUDY OF THE STRIATED AND GRANULAR DUCTS IN MOUSE AND RAT MANDIBULAR GLANDS

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Abstract. The mandibular gland in rodents presents a distinct type of ducts, granular, which are not encountered in other mammal species. Our aim was to conduct a stereological study in order to compare the number of striated and granular ducts on the section surface, and also the total surface occupied by each type of duct in part, between albino laboratory mouse and Wistar rat. Thus, we utilized 5 adult males from each species, harvested the mandibular glands and processed them for histological examination. The counts and measurements were performed with the aid of AmScope program, on a surface of 1699510 µm^2 for each subject in part and for the statistical analysis we used GraphPad Prism 6.01 software. The results showed that both species contain striated and granular ducts, but differently represented. The albino laboratory mouse presented a significantly smaller number of striated ducts in comparison to Wistar rat, and the surface occupied by these was significantly more reduced. Concerning the granular ducts from mouse mandibular gland, they were significantly better represented in albino laboratory mouse than in Wistar rat, occupying a significantly extended surface. The study highlights the importance of the secretion of granular ducts from albino laboratory mouse and Wistar rat mandibular glands.

Keywords: granular, mandibular, mouse, rat, striated

INTRODUCTION

The ductal system from rodent mandibular glands branches in intercalated, granular, striated, excretory and main excretory ducts (Amano *et al*., 2012). At birth, the gland is immature, containing solely transitory structures. Moreover, the granular ducts are absent, appearing after the age of one month, and subsequently becoming the best represented ducts. There are two growth and maturation phases for the gland: acinar and ductal phases (Jacob and Leeson, 1959; Coire *et al*., 2003). The acinar phase unreels in the first weeks of life, followed by the ductal one, when the granular ducts emerge and mature. In rat, the ductal phase takes place between days 28 and 96 of life, while in mouse, it takes place until day 35 of life (Pardini and Taga, 1997).

The glandular volume increase takes place between the first and third month of life (Coire *et al*., 2003), based on both the cellular volume and numeric increase. The differentiation and development of granular ducts cells occurs based on the cells lining the striated ducts (Gresik, 1994; Amano *et al*., 2012), but also through mitotic activity and proliferation of intercalated duct cells, migrated and differentiated in granular duct secretory cells (Srinivasan and Chang, 1975; Zajicek *et al*., 1985; Denny *et al*., 1993). These processes are dependent on testosterone levels (Pinkstaf, 1980; Amano *et al*., 2012). Thus, the mandibular gland in rodents displays sexual dimorphism, being larger in males than females (Frith and Townsend,1985), and the granular ductal system occupies less in females than in males (Amano *et al.,* 2012). The sexual dimorphism of the mandibular gland is more obvious in mouse than in rat (Amano *et al*., 2012).

Scientific literature mentions that after comparing approximately 300 species, no single description of salivary glands can be applied to all mammal species (Phillips, 1996; Tandler *et al*., 2001). The lack of conservation of the salivary glands structure is significant because it shows their evolution is not random, but correlates with their way of feeding or the environment in which they live (Phillips, 1996; Tandler *et al*., 2001). Thus, we aimed to conduct a stereological study in order to compare the number of striated and granular ducts on the section surface, as well as the total surface occupied by each type of duct in albino laboratory mouse and Wistar rat.

MATERIAL AND METHOD

The biological material was represented by 5 albino laboratory mice and 5 Wistar rats, males with an average weight of 37.5 g and 285 g, respectively. The study, was approved by the Bioethics Committee from University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca. After sacrificing the animals through prolonged exposure to isoflurane, we harvested the mandibular glands and fixed them in 10% buffered formaline. The fixation period was 7 days and the solution was renewed 3 times during this interval. After fixation, we dehydrated the sample with ethanol (increasing concentration: 70^0, 96^0, absolute), clarified it with n-butanol and embedded it in paraffin. The sample was sectioned, using a Leica rotary microtome, at a 5 µm thickness and the sections were contrasted utilizing Goldner's trichrome staining procedure.

Determination of striated and granular ducts surface

The sectioned samples were examined under an Olympus BX41 microscope and the images were captured with the attached camera (Olympus E-330), utilizing the 10x objective. We considered an equal surface (1699510 μm^2) for measurements, for all subjects taken into study. The duct count and measurement of the total surfaces of each duct in part were achieved with AmScope program. Thus, we determined the number of striated and granular ducts on the surface taken into study for each animal in part, the total surface occupied by each duct in part and expressed their procentual representation on the section surface.

Statistical analysis

The statistical analysis was made with the aid of GraphPad Prism 6.01 software, applying Unpaired t test. We compared the total average surfaces obtained for both striated and granular ducts, as well as the total number of striated and granular ducts/surface taken into study from the mandibular glands, between albino laboratory mouse and Wistar rat, and the level of significance was set at 5%.

RESULTS AND DISCUSSION

We can observe that the mandibular gland of both species taken into study contains both striated and granular ducts, and the number of granular ducts is markedly larger. They occupy a greater surface than the striated ones. By visually assessing the number of sections through granular ducts/surface taken into study, as well as the surface occupied by them in albino laboratory mouse (Fig. 1) and Wistar rat (Fig. 2), we can state that the albino laboratory mouse presents a lot more granular ducts, which consequently occupy a greater surface in the mandibular gland.

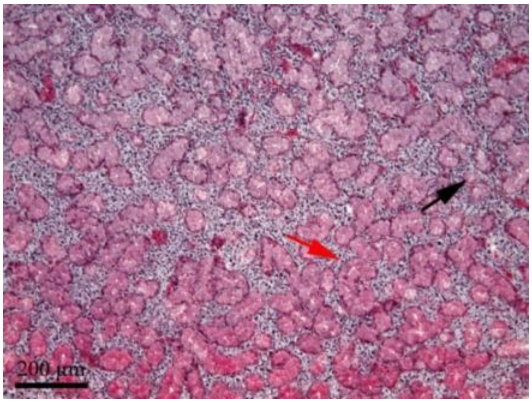

Fig. 1. Mandibular gland in albino laboratory mouse – striated ducts (black arrow); granular ducts (red arrow)

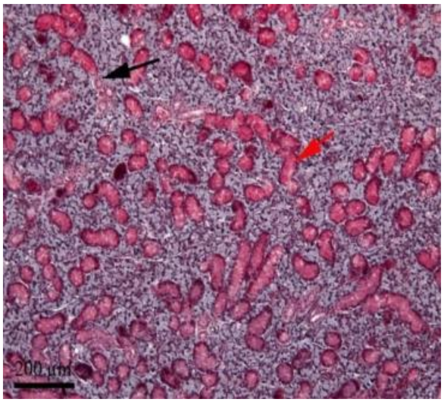

Fig. 2. Mandibular gland in Wistar rat - striated ducts (black arrow); granular ducts (red arrow)

The stereological analysis allowed us to quantify the number of striated and granular ducts and the total surface occupied by them for each animal taken into study and the results are presented in Table 1. After counting the striated ducts in mandibular gland the results indicated an average number of 6 striated ducts/surface taken into study for albino laboratory mouse and 49 striated ducts/surface taken into study in the case of Wistar rat. Regarding the average number of granular ducts/surface taken into study, it was 240 in the case of the mandibular gland in albino laboratory mouse and 187.4 granular ducts in Wistar rat, respectively.

The total average surface occupied by the striated ducts was 7446.73 µm^2 in the mandibular gland of the albino laboratory mouse and 77064.40 µm^2 in the mandibular gland of the Wistar rat. The situation differs in the case of the total average surface occupied by granular ducts, being larger in the albino laboratory mouse mandibular gland (1006260.67 µm^2) in comparison to the one in Wistar rat (521642.51 µm^2).

Table. 1.

Number of ducts/picture and total surface of ducts

Species	No. of striated ducts/picture	Total surface of striated ducts (µm^2)	No. of granular ducts/picture	Total surface of granular ducts (µm^2)
Mouse 1	4	4680.07	262	1011986.06
Mouse 2	7	7055.41	254	1006633.53
Mouse 3	4	3258.76	258	1054527.07
Mouse 4	8	9216.24	210	943109.68
Mouse 5	9	13023.16	218	1015047.00
Rat 1	47	79687.79	190	533882.26
Rat 2	49	94648.27	163	505010.83
Rat 3	69	81574.31	199	467949.54
Rat 4	49	80596.77	185	585047.00
Rat 5	32	48814.86	200	516322.93

Striated ducts occupy 0.44% of the total surface taken into study in the mandibular gland in albino laboratory mouse and 4.53% in Wistar rat, and granular ducts 59.21% from the surface taken into study in mouse and 30.69% in rat, respectively. These procentual representations are presented in Chart 1.

In other words, the striated ducts in albino laboratory mouse mandibular gland occupies a 10.3 times smaller surface than the one in Wistar rat, instead the granular ducts occupy 1.9 times more surface in the mandibular gland in albino laboratory mouse than in Wistar rat.

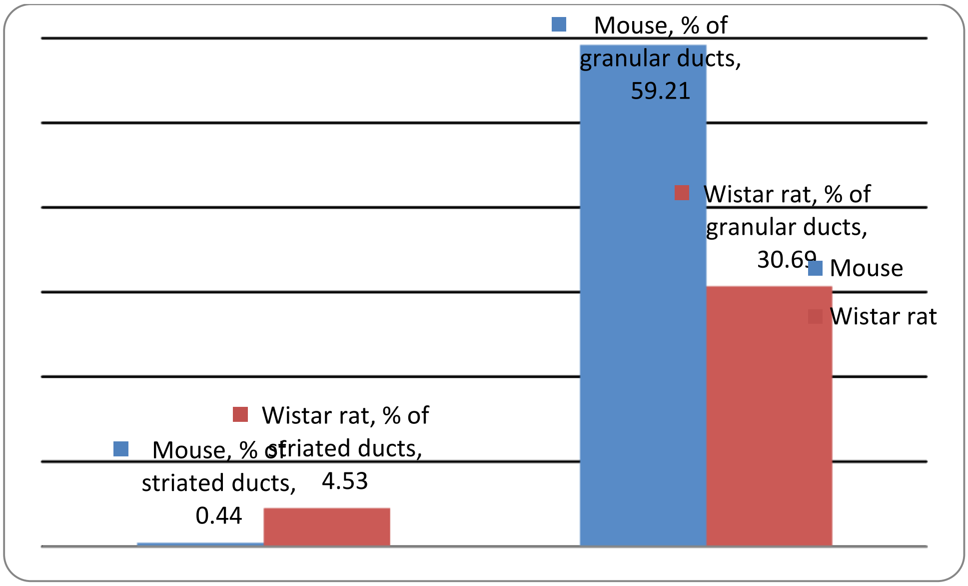

Chart 1. Percentage of intralobular ducts in albino laboratory mouse and Wistar rat

The results of the statistical analysis revealed that the differences regarding the number of ducts (striated and granular), as well as the total surface occupied by them in the mandibular gland in the two studied species were very significant ($p < 0.01$). This aspect indicates the fact that the mandibular gland in albino laboratory mouse contains significantly less striated ducts that the one in Wistar rat. However, the mandibular gland of albino laboratory mouse presents significantly more granular ducts than the on in Wistar rat.

At birth, the granular ducts are absent from the mandibular gland, maturing later in both mice and rats. The mandibular gland increases in volume based on the division of cells, but also on their increase in volume. The authors mention that this increase in volume can be due both to the accumulation of secretion granules, but also to the rough endoplasmic reticulum, which participates in forming the secretory products (Coire *et al.*, 2003). They studied the increase in volume of the acinar cells and of the cells lining the granular ducts in rat mandibular gland in a stereological study. They observed that the cells from granular duct increased in volume by 132% between day 28 and 96 of life (Coire *et al.*, 2003). The aspect suggests that these ducts become well represented in adults. The Wistar rats from our study were adult males and the granular and striated ducts are encountered in fairly large numbers on the surface taken into study (on average 49 striated ducts and 187.4 granular ducts), occupying a significant surface (4.53% for striated ducts and 30.69% for the granular ones from the total surface taken into study), while in albino laboratory mouse they are very well represented in the mandibular gland, exceeding even the surface occupied by the acini (59.21% from the total surface taken into study). This aspect suggests the importance of the granular ducts and the secretory products from the cells lining them for the species taken into study, particularly for the mouse.

The secretory cells are principal cells and their granules contain biologically active polipeptides exocitated during neural and hormonal stimulation (Amano *et al*., 2012). The other type of cells are pillar cells, with an approximately trapezoidal shape (with a narrower apical pole and wider basal pole), which nest between the principal ones. Their function is uncertain, but Amano *et al.* (1993) suggested a paracrine function.

The importance of the secretion of cells from granular ducts for the two studied species is mainly suggested by the large quantity of intracytoplasmatic granulations and secondly by the size of the cells which appear much taller than the ones from the acinar structure or striated ducts. Another aspect is linked to the length of the granular ducts and their sinuous aspect, which are also directly linked to the importance of the secretion of the cells from granular ducts in mouse and rat.

In the scientific literature, the researchers specify the fact that the sexual dimorphism of the mandibular gland in mouse is clearly more obvious than than the one in rat (Amano *et al*., 2012). In our study, we observed the fact that the granular ducts in albino laboratory mouse are much better represented than in Wistar rat, both numerically and regarding the occupied surface.

In other stereological studies, carried out in mice, the authors mention that females have fewer secretory granules than males and that the granular duct system in female mandibular glands occupies 19-36%, while in male it occupies 47-65% (Gresik, 1994). All utilized mice in our study were adults. Similarly, in our study, albino laboratory mice males contained well represented granular ducts in the mandibular gland, occupying on average 59.21% from the total surface taken into study.

Our study indicates the fact that in albino laboratory mouse and Wistar rat, the striated and granular ducts have an important role, reflected by the number and large surface occupied by them. Thus, the substances secreted by this type of ducts significantly participate in the digestion process as well as the defense ones for this species which live in very particular conditions.

CONCLUSIONS

The granular ducts are significantly more numerous compared to the striated ones in both studied species. The interspecific differences regarding the number and surface occupied by the granular ducts were very significant in the favour of mouse, aspect that would suggest the different importance of the cellular secretory product from granular ducts in albino laboratory mouse mandibular gland and Wistar rat, respectively.

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