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Histomorphometrical study of the submandibular gland ductal system in the rat

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Summary. The duct system of murine submandibular gland is composed, in contrast with other mammals, by four types of ducts, among which the granular duct is unique for rodents. The granular duct shows a typical secretory structure with a clear intersex morphological diversity on which we carried out a morphometrical study in order to determine the relative area of each duct in rats in comparison with the rest of ducts and the whole gland. Our results, in both sexes, show that the duct with the broadest surface is the granular duct, followed by the excretory, striated and the intercalated ducts. In addition, we found a significant intersex difference between the relative surface of the granular and the excretory ducts, being bigger in males than in females. Finally, in both sexes, there is a greater variation in the data related to the excretory ducts than to the other ducts.

Key words: Submandibular gland, Morphometry, Ductal system, Histology

Introduction

The peculiar ductal system of the submandibular gland is well known in rodents and particularly in rats, in relation to other mammals. In rats there is a unique duct denominated "*the granular duct*", located between the intercalated and the striated ducts. Therefore, the whole duct system of the submandibular gland in rodents is composed, starting from the Secretory Terminal Unit (STU), by the following ducts: intercalated, granular, striated and excretory (Shackleford and Wilborn, 1968; Cutler and Chaudhry, 1975; Brocco and Tamarin, 1979; Pinkstaff, 1979; Brunel and Colin, 1987).

Considering, the existence of the granular duct in rodents, with clear secretory functions, such as the production of the epithelial and nerve growth factors (EGF and NGF), (Danz et al., 1999; Tajina et al., 2000), and the sexual diversity related with the morphology of this duct (Pinkstaff, 1998), we found it interesting to perform a study comparing the relative importance of each duct within the gland, given that we have found few references to this issue. Only the work by Tamarin and Sreebny (1965), who carried out a morphometric study of the submandibular gland in seven rats, without sex distinction, takes into account the volume of the ducts or their proportions as the main variable. There is another morphometric study by Gutierrez et al. (1990), focused on cellular measures and secretion granules of the granular duct. Accordingly, the present study deals with a morphometric approach to show the relative weight between these ducts and in relation to the whole gland. Other morphometric studies we can mention focused to detect the sexual dimorphisms in rodents, are Jayasinghe et al. (1990) who studied mice and Rins de David et al. (1990) who used rats.

Materials and methods

Submandibular glands were obtained from fifty 16-week-old Wistar rats, twenty-five males and twenty-five females. One gland per animal was used for morphometric analysis. The animals were anesthesized intraperitoneally, before the animal's death the glands were removed, then fixed in neutral formalin and embedded in paraffin. Serial 7 μm slices were stained with Hematoxylin-Eosin. We carried out a morphometric study of the submandibular gland ductal system in rats in these slices following the method described below.

We studied one of ten slides from each gland where we chose ten squared areas of 107672.16μm^2, distributed on the surface of each cut, according to the following method: four equidistant fields were selected on the longitudinal axis and another six on two parallel axis to the previous one, three fields on either side, mutually equidistant and equally distant from the longitudinal axis and from the lateral boundary of the slide.

All the ducts contained in each of these areas were

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measured and classified into four types: intercalated, granular, striated and excretory. We measured the following variables in each one: area, relation of whole area of each field/area of duct expressed in percentage; and relation of whole area/sum of the areas of each type of duct expressed in percentage. Furthermore, for all structures of each class in the same area, and for each one of the above mentioend parameters, the sum, the mean and the standard deviation were calculated; this last was also done for each duct measured in the whole gland.

All the aforementioned was carried out with the aid of VIDS IV software by Analytical Measuring Systems (AMS), and implied the measurement of an average of 87 intercalated, 1318 granular, 213 striated and 89 excretory ducts per each male gland, and 66 intercalated, 921 granular, 141 striated and 35 excretory ducts of per each female gland.

Thereafter, two statistical studies of the data were carried out: The Anova Test and the Mean Comparison Test of Bonferroni, using the statistical analysis program R-SIGMA .

Results

After obtaining the data from each experimental animal, the mean of each duct's surface percentage related to the total surface of the studied area was calculated. We therefore had 25 numerical data for each studied duct type per sex. This implies that we had eight data groups for which we carried out the statistical study, each of these data figures being the mean of the each duct's surface percentage in relation to the total surface of the microscopic field for a specific experimental animal.

Considering the statistical study of these data we obtained the values expressed in table 1 designating the relative percentage means with respect to the whole area studied, of each conduct type in each sex.

A test of comparison between both sexes for each duct type was carried out, which demonstrated that there were no significant differences between the relative areas of the intercalated ducts (p=0.566) and the striated ducts (p=0.257) of either sex. On the other hand, there were significant differences between the relative areas of the granular (p=0.00004) and excretory ducts (p=0.0003) of males and females. The dispersion of the values obtained from the excretory ducts of both sexes was also taken into account, being higher in female rats.

All the data are shown in figures 1 to 5. Figures 1 and 2 show the same relationship, but in this case taking into account the other structures of the gland, which are labeled as "remaining tissue" and that are obviously the secretory terminal units and stroma. Figure 3 shows the comparison among the relative area of all the ducts for each sex. And finally, Figures 4 and 5 show the comparison of the dispersion between the granular duct (Fig. 4) and the excretory duct (Fig. 5) of both sexes, since, as we have already commented, there was a significant difference between them.

Discussion

The relative importance of each duct in relation to

Table 1. Mean distribution and standard deviation between the averages of percentages of the four ducts in male and female animals.

	INTERCALATED DUCT	GRANULAR DUCT	STRIATED DUCT	EXCRETORY DUCT
Male	1.2284±0.599	21.1940±1.225	2.0828±0.403	8.3088±1.979
Female	1.3284±0.627	19.3936±1.569	2.2048±0.347	6.0168±2.186

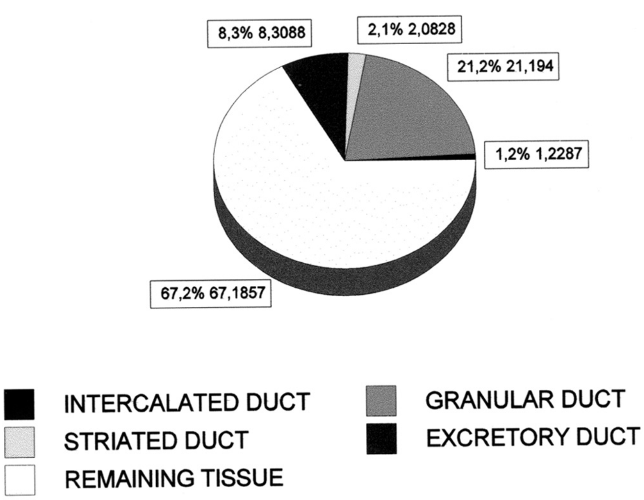

Fig. 1. Diagram that shows the proportions among the areas of the ducts from the male rat.

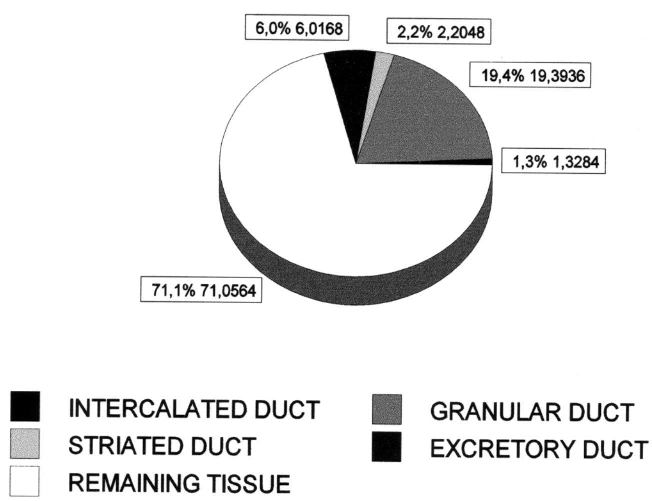

Fig. 2. Diagram that shows the proportions among the areas of the ducts from the female rat.

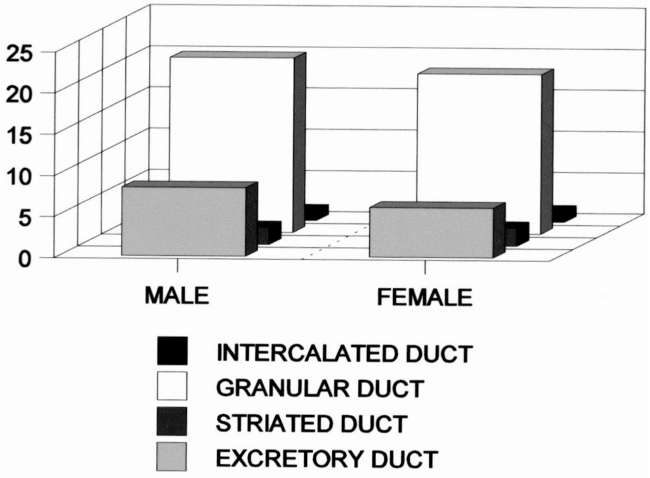

Fig. 3. Diagram that shows the comparison among the relative area of all the ducts for each male and female rat (y axis in %).

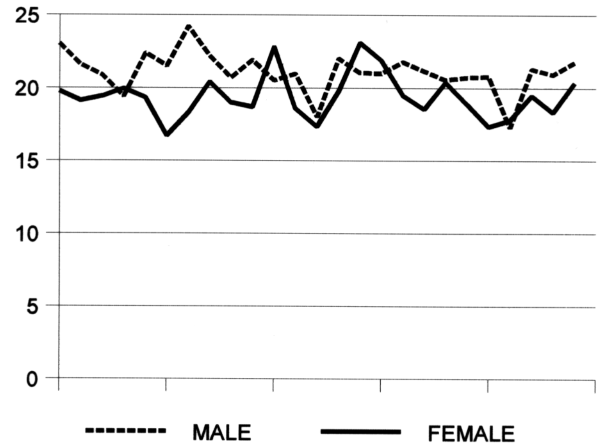

Fig. 4. Comparison of the dispersion among the granular ducts in male and female rats (y axis in %).

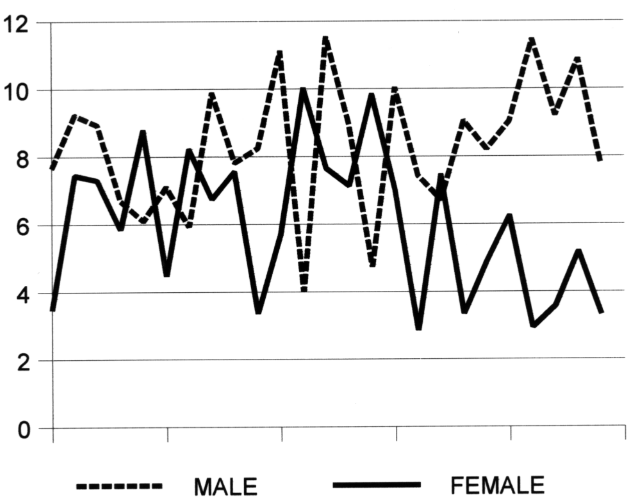

Fig. 5. Comparison of the dispersion among the excretory ducts in male and female rats (y axis in %).

all ducts and to the total surface must be highlighted in the morphometric results. The granular duct, in males as well as in females, had without doubt the broadest surface, with values of 21.19% in males and 19.39% in females, followed at a distance by the excretory duct, yielding values of 8.90% in males and 6.01% in females. Falling behind, we find the values of the other two ducts, the striated duct being 2.08% in males and 2.20% in females, while the intercalated duct was 1.22% in males and 1.32% in females.

Concerning the comparison between sexes, we found that the intercalated duct and the striated duct did not differ significantly, while there were significant differences between the granular and excretory duct in males and females, the percentages in males, as mentioned before, being higher. In the case of the granular duct, there were significant morphological differences between sexes, as has been numerically demonstrated. The possible explanation, in the case of the excretory duct, is that this organ being bigger in males, and this bigger excretory duct being located in a higher proportion in the central zone, closer to the hilus, aleatoriously, more fields with this type of duct were observed, taking into account that one only one of these can occupy a great part of the miscroscopic field. This could explain the high data dispersion for this duct in both sexes.

Comparing our data with those by Tamarin and Sreeby (1965), performed in rats, we conclude that the divergence exists. These authors reported a volume mean of 4% for the intercalate duct (higher than our results); 18% for the granular duct lower than our results); 1% for the striated duct (lower than our results); and they do not give figures for the excretory duct, since they only measured the intralobular tissue, and they reported having found great dispersion in their data. The measurements they refer to are volumetric and not surface, and they only studied seven glands without sex distinction. On the other hand, as far as the sex diversity is concerned, this was demonstrated in mice by Jayasinghe et al. (1990) among others, and our conclusions regarding a higher relative surface of the granular duct in males agree with Rins de David et al. (1990) who carried out a study on rats.

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