POLYMORPHISM OF THE ACINI IN SUBLINGUAL GLAND IN SOME SPECIES OF RODENTS - MORPHOLOGICAL STUDY

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Abstract. Salivary glands are a feature of terrestrial species, their structure being different depending on the species and diet. In special literature we did not found informations about the acini polymorphism in sublingual gland, therefore we consider it appropriate to do a morphometric study in some rodents. In this study, we harvested the sublingual glands of three males of the following species: guinea pig, Wistar rat, mouse, rabbit and chinchilla. The pieces were histologically processed in order to evaluate them. For for measuring and counting the acini, we used AmScope software. The results emphasize that the highest number of acini is found in mouse, followed by chinchilla, Wistar rat, rabbit and then guinea pig. Regarding the dimensions, the biggest acini are found in guinea pig, being also the most polymorphic ones, followed by rabbit, Wistar rat, chinchilla and the smallest acini are found in mouse. We did not find any statistically significant differences between the size of the acini in rabbit with guinea pig and chinchilla with Wistar rat, but when comparing guinea pig with Wistar rat, chinchilla and mouse, and also when comparing rabbit with Wistar rat and mouse, we found that there were highly statistically significant differences. When comparing the rabbit with chinchilla, recorded values were statistically significant.

Keywords: acini, morphometry, rabbit, rodents, sublingual.

INTRODUCTION

Salivary glands are present in a large number of animal species, from nevertebrates to vertebrates. Their degree of complexity is very different from one species to another, depending on a number of factors, including: class, gender, species and characteristics of the alimentation (Da Cunha Lima *et al*., 2004; Gresik, 1994). The composition of saliva is also dependent on the nature of the food. For example, in rodents, secretion of the saliva is adapted to dry food, in ruminants, the parotid secretion is predominantly rich in water, and carnivores present predominantly mandibular secretion, rich in mucin filaments. In omnivores, the discharge varies depending on the food (Gresik, 1994; Tache, 1994). The major salivary glands category includes: parotid, mandibular and sublingual glands. Both their size, the structure of the acini and the features of saliva secreted (serous, mucous or mixed), varies from one species to another and is influenced by the diet (SissoN and Grossman, 1964; PopovicI *et al*., 2003; Treuting and Dintzis, 2012). Sublingual glands are the smallest of the major salivary glands in rodents (Tucker and Miletich, 2010). These glands are classified as mixed tubuloacinar, the majority of the secretory units being mucous, surrounded at the periphery by several serous cells, forming the serous demilunele. Sublingual gland in mice and Wistar rats consists of mucosal tubuloacinar structures, showing relatively little serous demilunes. Ultrastructural studies on prenatal development of sublingual gland in mice and Wistar rats have shown that the development of mucous and

serous acinar cells differ in some aspects among animal species (Gresik, 1994; Taga and Sesso, 1998). Since we did not found in special literature information about acini polymorphism in the sublingual gland in mammals, we considered it appropriate to do a morphometric study in some of the rodents.

MATHERIALS AND METHODS

The biological material used in this study was represented by three males of the following species: guinea pig (*Cavia porcellus*), Wistar rat (*Rattus norvegicus*), mouse (*Mus musculus*), rabbit (*Oryctolagus cuniculus*) and chinchilla (*Chinchilla laniger*). The animals were euthanized by prolonged exposure to inhalational anesthesia (Isoflurane). Sublingual glands were harvested for histological and morphometric investigations. The harvested pieces were fixed in 10% formalin, dehydrated in ethyl alcohol (70º, 95°, absolute), clarified with n-butanol and embedded in paraffin. Sections of 5μm thickness were stained with hematoxylin-eosin and examined with an Olympus BX41 optical microscope, equipped with digital camera. For the investigation, we used AmScope program, taking into study a 1699509.677 μm^2 surface. The acquired data was analyzed with GraphPad Prism 6 software. We determined the following values: minimum, maximum, mean, standard error of mean and standard deviation. We also calculated the percentage occupied by acini out of the surface taken into study. The difference in area was occupied by other structural elements (excretory ducts, other types of acini, connective tissue, blood vessels).

RESULTS AND DISCUSSIONS

In sublingual gland of mice, the predominant acini are the mucous ones. They are very polymorphic and have different sizes (Fig. 1). Sublingual gland in Wistar rats looks similar to the one in mice, containing very polymorphic acini and having different sizes (Fig. 2). In rabbits, the general appearance of the sublingual gland is comparable to the one in Wistar rats indicating that here, the polymorphism of the mucous acini is more pronounced (Fig. 3). In guinea pigs, sublingual gland differs from the species presented above. In addition to being highly polymorphic, the sublingual mucous acini from guinea pigs are clearly larger than the other species. Morphologically, these acini secrete mucus, but they are obviously higher (Fig. 4). In chinchillas, the sublingual gland is similar to the one in Wistar rats in terms of general aspect, but there are some differences too - the acini are comparable in size and polymorphism, but has a wider lumen (Fig. 5). Morphometrically, this investigation revealed that on the same area of section, the number of acini is different from one species to another. The highest number of the acini/ studied surface was found in mouse (333), followed by chinchilla (220), Wistar rat (209), rabbit (131) and the lowest in guinea pigs (101) (Table 1).

The areas occupied by the acini out of the total surface taken into the study (1699509.677 μm^2) are comparable, but not identical. The highest and closest values were recorded in guinea pigs (1,447,478.11 μm^2) and mouse (1,447,507.37 μm^2, representing 85.17%), followed by Wistar rats (1,436,390.67 μm^2, representing 84.52%), chinchilla (1,417,531.45 μm^2, meaning 83.41%) and the lowest values were in rabbit (1,403,188.59 μm^2, 82.56%). These differences are not very major, even if the number of the acini on the surface section differs greatly from one species to another. Thus, in guinea pigs and mice, the surface occupied by acini is comparable, although the number of acini on the same area is very different (101 to 333). The fact that 101 acini occupy the same surface as 333 acini

is because in mouse, the number of acini/total surface is 3.29 times higher than in guinea pig. The difference is given by the higher average size of the acini in guinea pig compared to mouse.

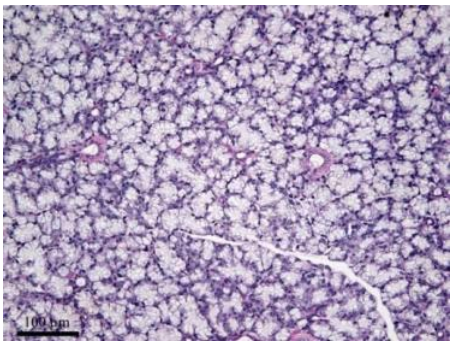


Fig. 1. Sublingual gland in mouse (H-E)

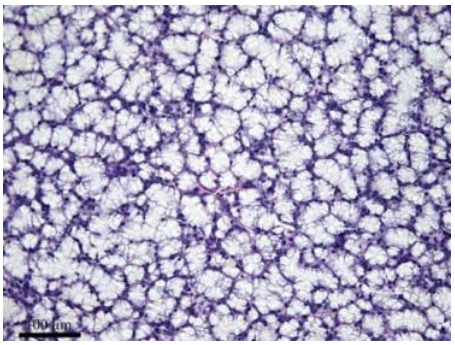


Fig. 2. Sublingual gland in Wistar rat (H-E)

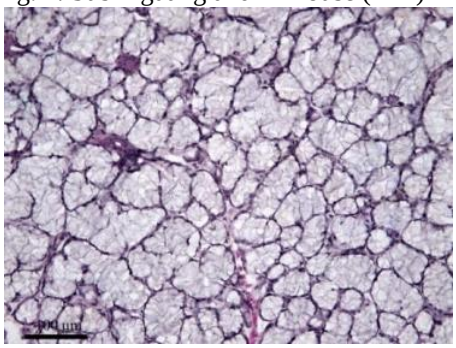


Fig. 3. Sublingual gland in rabbit (H-E)

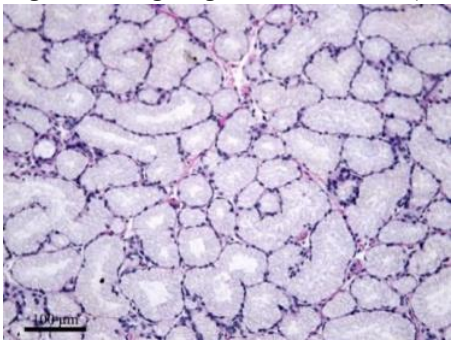


Fig. 4. Sublingual gland in guinea pig (H-E)

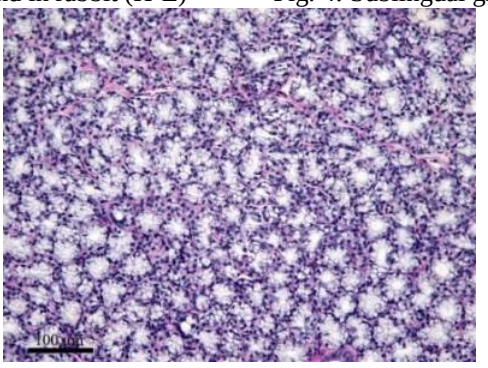


Fig. 5. Sublingual gland in chinchilla (H-E)

Table 1

Number of the acini/studied surface (1699509.677 μm^2)

	Cavia porcellus	*Oryctolagus cuniculus*	*Rattus norvegicus*	*Chinchilla lanigera*	*Mus musculus*
N=	101	131	209	220	333
Mean	198,8				

The dimension of acini was assessed by measuring their surface section at all acini on the total area taken into study. Thus it could be appreciated both acini size and their polymorphism. We detected some differences between the species studied by us, both small and significant ones. The highest mean value was found in guinea pigs (14331 μm^2),

followed by rabbits (10711 μm^2), Wistar rats (6873 μm^2), chinchilla (6443 μm^2) and the lower value was found in mice (4347 μm^2) (Table 2). These numbers clearly show that in sublingual gland, the biggest acini are found in guinea pigs and the smallest ones in mice. If we compare the average size of the acini from guinea pig to that of the other species, we find that it is 1.33 times bigger than the one in rabbit, 2.08 times bigger than the one in Wistar rat, 2.22 times bigger than the one in chinchilla and 3.29 times bigger than the one in mouse.

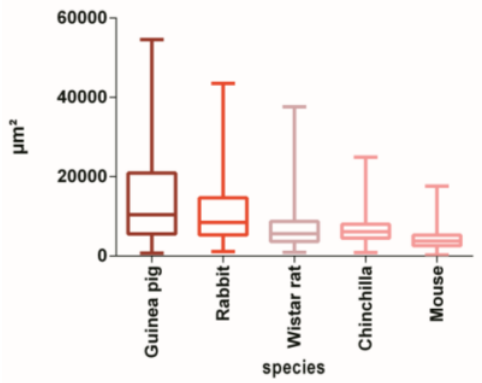
Table 2

Dimensions of the acini. Min – minimum; Max – maximum; $\bar{x}$ - mean; SD – standard deviation; SEM – standard error of mean; CV - Coefficient of variation

Picture surface μm² 1699509.677 μm²					
	Cavia porcellus	*Oryctolagus cuniculus*	*Rattus norvegicus*	*Chinchilla lanigera*	*Mus musculus*
Min (*μm²*)	716.8	1147	900.8	866.9	292.7
Max (*μm²*)	54585	43543	37638	24943	17637
$\bar{x}$ (*μm²*)	14331	10711	6873	6443	4347
SD (*μm²*)	11901	7828	5065	3175	2605
SEM (*μm²*)	1184	683.9	350.4	214.1	142.8
CV (%)	83.04	73.08	73.70	49.28	59.93

Table 3

Minimum, maximum and mean values of the acini surface



Regarding the coefficient of variation, the highest value is in guinea pig with 83.04% (minimum 716.8 μm^2, maximum 54585 μm^2, mean 14331 μm^2), followed by Wistar rat with 73.70% (minimum 900.8 μm^2, maximum 37638 μm^2, mean 6873 μm^2), rabbit 73.08% (minimum 1147 μm^2, maximum 43543 μm^2, mean 10711 μm^2), mouse 59.93% (minimum 292.7 μm^2, maximum 17637 μm^2, mean 4347 μm^2) and the lowest in chinchilla, 49.28% (minimum 866.9 μm^2, maximum 24943 μm^2, mean 6443 μm^2) (Table 3). The smaller the coefficient of variation is, the less polymorphic the acini are. Assessing the acini polymorphism of the sublingual gland in the species studied by us, we found that the most polymorphic ones are found in guinea pig, followed by Wistar rat, rabbit, mouse, and the less polymorphic acini are found in chinchilla. In terms of multiple comparison test, there were no statistically significant differences between the size of the acini in rabbit with guinea pig and chinchilla with Wistar rat. To analyze the results of statistical calculations, we used one-way ANOVA test. When comparing guinea pig with Wistar rats, chinchilla and mouse, we found that there were highly statistically significant differences ($P < 0.0001$). We obtained

the same results by comparing the rabbit with Wistar rat and mouse, the mouse with Wistar rat and the mouse with chinchilla. When comparing the rabbit with chinchilla, recorded values were statistically significant (P <0.001) (Table 4).

Table 4

Results of the statistical analisys for the studied species

Multiple comparison test	Statistical significance
Rabbit vs. Guinea pig	ns
Wistar rat vs. Guinea pig	****
Chinchilla vs. Guinea pig	****
Mouse vs. Guinea pig	****
Wistar rat vs. Rabbit	****
Chinchilla vs. Rabbit	***
Mouse vs. Rabbit	****
Chinchilla vs. Wistar rat	ns
Mouse vs. Wistar rat	****
Mouse vs. Chinchilla	****

The statistical results attest the existence of some major differences regarding the size of the sublingual gland acini in the species studied by us.

CONCLUSIONS

Regarding the investigation of the sublingual gland acini in the species studied by us, we can affirm that the polymorphism is pronounced. Among the species taken into study, the most polymorphic acini are in guinea pigs and the least polymorphic in chinchilla. The highest average surface in acini was noticed in guinea pigs and the smallest in mice. Mouse has the numerous acini/studied surface, followed by chinchilla, rat, rabbit and guinea pig.

REFERENCES

1. Da Cunha Lima Marta, D. Sottovia-Filho, Tania Mary Cestari, R. Taga, 2004, Morphometric characterization of sexual differences in the rat sublingual gland, Braz Oral Res. 18(1):53-8.
2. Gresik E.W., 1994, The granular convoluted tubule (GCT) cell of rodent submandibular glands. Microscopy Res Tech 27:1–24.
3. Popovici I., A. Damian, N. Popovici, Ioana Chirilean, 2003, Tratat de anatomie comparată. Splanhnologie, Editura AcademicPress, Cluj-Napoca.
4. Sisson S., J.D. Grossman, 1964, The anatomy of the domestic animals, Fourth Edition, Revised, W.B. Saunders Company, Philadelphia and London.
5. Tache Simona, 1994, Fiziologia glandelor salivare, Editura Dacia, Cluj-Napoca.
6. Taga R., A. Sesso, 1998, Postnatal development of the rat sublingual glands. A morphometric and radioautographic study, Arch. Histol. Cytol., 61(5):417-426.
7. Treuting P.M., S. Dintzis, 2012, Comparative anatomy and histology. A mouse and human atlas, Academic Press, Washington.
8. Tucker Abigail S., Isabelle Miletich, 2010, Salivary glands- development, adaptations and disease, London, Editura Karger, Front Oral Biol. Basel, Karger, vol 14, pp 1-20.