Comparative assessment of the sublingual secretion type in rabbit and some rodent species. Histochemical study.

BIANCA MATOSZ (1), FLAVIA RUXANDA (1)*, GABRIELA MUȚIU (2), ADRIAN GAL (3), CRISTIAN RAȚIU (4), RĂZVAN VICAȘ (5), VIOREL MICLĂUȘ (1)
1 Department of Histology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
2 Department of Histology, Faculty of Medicine and Pharmacy, University of Oradea, Romania
3 Department of Pathologic Anatomy, Necropsy and Veterinary Forensic Medicine, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
4 Department of Dental Medicine, Faculty of Medicine and Pharmacy, University of Oradea, Romania
5 Department of Anatomy, Faculty of Medicine and Pharmacy, University of Oradea, Romania
*Corresponding author
Flavia Ruxanda, Ph.D. Departments of Cell Biology, Histology and Embryology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăștur St., Cluj-Napoca 400372, Romania Phone: +40(0)264.596.384/192, Fax: +40(0)264.593.792 E-mail: flavia.ruxanda@gmail.com

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Summary

Salivary glands are adapted to the necessities of each species. In this context, we conducted a comparative histochemical study on the sublingual gland in rabbit and three rodent species, assessing the secretion type. We used the following species: mouse, guinea pig, chinchilla and rabbit. We harvested the sublingual glands and histologically processed them. The samples were subdued to PAS reaction to highlight neutral mucosubstances and Alcian blue to identify acid and sulfated mucosubstances. In mouse, guinea pig and chinchilla, the cytoplasm of the acini was intensely PAS positive. In rabbit, cells in acini presented a PAS negative reaction. Alcian blue reaction turned out to be positive in all species taken into study, with significant differences from one species to another. The differences among the rodents were represented by the number of positive cells and the reaction intensity. In rabbit, the situation was different in comparison to the rodents, with an intensely positive Alcian blue reaction. The secretion of the sublingual gland in rabbit differs from all other rodent species taken into study, containing exclusively acid and sulfated mucosubstances. In rodents, the sublingual gland synthesizes large quantities of neutral mucosubstances, mall or at most moderate quantities of acid and sulfated mucosubstances.

Introduction

Rodents fall under Animalia reign, Chordata phylum, Mammalia class and Rodentia order. Although it resembles rodents from many points of view and belongs to the same reign, phylum and class, the rabbit belongs to a different order: Lagomorpha. Because of this, the rabbit is not considered a rodent. This is due to the fact that from an anatomical point of view, leporids have a number of 6 incisor teeth, unlike rodents, which possess 4 incisor teeth (Weisbroth et al., 2013). The additional pair of incisors is reduced in size and is situated immediately behind the incisors on the superior maxilla (Alvarez-Castaneda et al., 2017). These small teeth are rounded and lack the sharp edge. They are visible only if
the oral cavity is opened and if we check behind the superior incisors. *Lagomorpha* order comprises two major families: *Leporidae* (rabbits and hares) and *Ochotonidae* (pikas), with numerous genera and species from all over the world (Hoffman and Smith, 2005; Buseth and Saunders, 2014). The main genera of leporids include *Lepus* genus (hares), *Oryctolagus* rabbits) and *Sylvilagus* (cottontails) (Weisbroth et al., 2013; Ge et al., 2013; Ge et al., 2015). Each species has certain particularities linked to the environment they live in and alimentation type they have, and salivary glands are structures adapted to the necessities of each species in part. Anatomically, the rabbit is not considered a rodent species, although it resembles most of them. In this context, we considered as opportune to conduct a comparative histochemical study on the sublingual gland in rabbit and three rodent species, in order to assess if there are major differences among them concerning the structure and secretion type.

**Material and methods**

The biological material utilized in this study belongs to three species from *Rodentia* order (mouse, guinea pig and chinchilla) and one species from *Lagomorpha* order (rabbit). We utilized three animals of each of the following species: mouse (*Mus musculus*), Guinea pig (*Cavia porcellus*), chinchilla (*Chinchilla lanigera*) and rabbit (*Oryctolagus cuniculus*). We harvested the sublingual glands and histologically processed them. The samples were fixed in 10% buffered formalin for 7 days and the fixation solution was renewed three times. We then dehydrated the sublingual gland tissue samples in ethanol, clarified them in n-butanol and embedded them in paraffin. We sectioned the tissue at 5 μm thickness with the aid of a Leica rotary microtome and subdued the obtained slides to PAS and Alcian blue reactions. PAS reaction was used in order to highlight neutral mucosubstances and Alcian blue to identify acid and sulfated mucosubstances. The histological slides were examined under an Olympus BX41 light microscope, endowed with a digital camera (Olympus E-330) for capturing images. We used Adobe Photoshop CS2 software in order to process the obtained images.

**Results and discussions**

In mouse, the sublingual gland is completely formed out of acini, whose cells present an intensely PAS positive reaction, without significant differences from one acinus to another or from one area to another (Fig. 1).

![Fig. 1. Sublingual gland in mouse – PAS reaction.](image)
Fig. 2. Sublingual gland in guinea pig – PAS reaction.

In chinchilla, the sublingual gland also contains acini formed out of cells with an intensely PAS positive cytoplasm (Fig. 3).

Fig. 3. Sublingual gland in chinchilla – PAS Reaction.

In rabbit, the general aspect of the studied salivary gland is completely different in comparison to the previously presented species in the sense that the cells from the glandular acini do not present PAS positive material, not even discrete one (Fig. 4). In mouse, the sublingual gland is Alcian blue positive, but uneven regarding the intensity of the reaction, from one acinus to another as well as from one area to another (Fig. 5).

Fig. 4. Sublingual gland in rabbit – PAS Reaction.
In guinea pig, the situation is very particular in the sense that there is a relatively small number of cells with intensely positive reaction, present only in some acini, where they can represent a small or large percentage. Sometimes, all the cells lining the acinus present intensely positive Alcian blue reaction (Fig. 6). In most of the acini, the reaction is mildly positive. In chinchilla, the situation is very particular, with vast areas presenting positive reaction and others with mildly positive or even negative reaction. The alternance of such areas can be observed even in the same lobule, but most of the times the reaction is different from one lobule to another (Fig. 7). In rabbit, the sublingual gland presents intensely positive reaction to Alcian blue, which is uniform throughout the whole section surface of the lobules (Fig. 8).
Discussion
The fact that the sublingual gland is the gland that secretes most of the salivary mucosubstances (Jacob and Poddar, 1978; Al-Saffar and Simawy, 2014) is well known. The primordial role of the saliva is protection of the oral cavity from mechanical, termic and chemical irritation and also lubricating it (Chiappin et al., 2007; Roussa, 2011; Edgar et al., 2012).

Neutral mucosubstances are highlighted through PAS reaction and the acid and sulfated ones with Alcian blue (Jacob and Poddar, 1978; Kiernan, 1990; Moghaddam et al., 2009; Al-Saffar and Simawy, 2014). Applying PAS reaction on sublingual sections highlighted both comparable aspects and significant differences among the species taken into study. The comparable aspects were noticed in the case of the three rodent species in which, the cytoplasm of the acinar cells appears to be filled with PAS positive material with a relatively uniform disposition in all cells from the lobules’ section surface. This aspect suggests an intense neutral mucosubstances synthesis activity in all cells from the sublingual gland in the three rodent species taken into study.

The situation is completely different in the case of the rabbit, in which PAS reaction was negative. This demonstrates that acinar cells from rabbit sublingual gland do not synthesize neutral mucosubstances, not even in small quantities. The fact that the secretion of the sublingual gland in rabbit is different from the one in the rodents from our study clearly stands out, at least concerning the neutral mucosubstances, absent in rabbit and present in large quantities in the other three rodent species taken into study. Alcian blue reaction for acid and sulfated mucosubstances was positive in all studied species, but with significant differences from one species to another. In rodents taken into study, although Alcian blue reaction is positive, there are differences among them regarding the number of cells engaged in acid and sulfated mucosubstances’ secretion, and also the intensity of their secretion. Thus, in mouse, sublingual gland is positive, with a mild intensity Alcian blue reaction, but nonuniform, with large differences between one acinus and another, especially between one area and another. It is worth noticing the zonal nonuniformity of the reaction, a somehow particular aspect either highlighting differences in the mucosubstances secreted by cells in acini from mouse sublingual gland, or suggesting the fact that the secretion was eliminated from some cells, acini or area, whereas it is still present in others. In guinea pig, there is a relatively small number of cells with intensely positive Alcian blue reaction, present only in some acini, where not necessarily all cells are positive. There are situations in which the positive cells represent a small or large percentage or others when all acinar cells are intensely positive to Alcian blue reaction. In the case of other glandular acini, which are as a matter of fact majoritary, Alcian blue reaction is mildly positive. We can affirm that the sublingual gland in guinea pig synthesizes acid mucosubstances in small quantities. In chinchilla, there are vast areas with positive reaction to Alcian blue, which alternate with areas presenting a mildly positive or even negative reaction. Such differences can be highlighted in the same...
lobule, but most of the times with average differences in intensity. The differences are very obvious regarding the reaction intensity from one lobule to another. We can remark the fact that Alcian blue reaction, although positive in all three rodent species, highlights differences regarding the number of the cells involved in the secretion of acid and sulfated mucosubstances. On the other hand, we noticed that in the studied rodent species, the quantity of acid and sulfated mucosubstances is significantly smaller in comparison to the neutral ones, highlighted through PAS reaction. In rabbit, the situation is completely different in comparison to the rodents taken into study, in the sense that here, the reaction to Alcian blue is intensely positive. Other authors also stated that sulfated mucosubstances behave in an entirely different way in the salivary glands of rabbits and rodents (Bondi et al., 1984). It is worth pointing out that in rabbit sublingual gland, all acinar cells from the lobule section surface have an intensely positive reaction to Alcian blue, without any differences from one acinus to another or one area to the other. The participation of all acinar cells to acid and sulfated mucosubstances’ secretion and the intensity of the reaction to Alcian blue present in all cells, clearly demonstrates the fact that rabbit sublingual gland synthesizes large quantities of such substances.

Conclusions

The secretion of the sublingual gland in rabbit is different from the ones in the rodent species taken into study, synthesizing exclusively acid and sulfated mucosubstances unlike the rodents, whose sublingual gland synthesizes large quantities of neutral mucosubstances and small or at most moderate amounts of acid and sulfated mucosubstances. The completely different secretion of the sublingual gland in rabbit in comparison to the other rodents suggests to a certain extent that the rabbit is not a typical rodent not only from an anatomical point of view.

References


