Annals of R.S.C.B., Vol. XXI, Issue 3, 2017, pp. 7 – 12
Received 14 September 2017; accepted 29 May 2017.
**doi:** 10.ANN/RSCB-2017-0016:RSCB

# Secretory mechanisms in granular ducts in rat mandibular gland

FLAVIA RUXANDA [(1)], GABRIELA MUȚIU [(2)], ADRIAN GAL [(3)], BIANCA MATOSZ [(1)*], CRISTIAN RAȚIU [(4)], VLAD-EMIL LUCA [(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 Surgical techniques, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania*
**Corresponding author*
*Bianca Matosz, Ph.D. student*
*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: bianca.matos@usamvcluj.ro*

**Key words:** apocrine, holocrine, granular, mandibular, merocrine.

## Summary

Granular ducts in the mandibular gland in rats present various types of cells. The secretion mechanism of these cells is different, thus we aimed to assess the secretory mechanisms present in cells lining the granular ducts in the mandibular glands in albino Wistar rat. The study was approved by the Bioethics Committee of University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca. We utilized 5 adult male albino Wistar rats, harvested the mandibular glands and processed them for histological examination. Upon examining the slides, we observed that most of the cells presented merocrine secretory mechanism, but some presented bulges in the apical pole with a tendency to strangle and detach as vesicles, signs of apocrine secretory mechanism. We also identified cells with discontinuities in the apical pole, with a tendency to eliminate all the cytoplasmic components. The aspect suggested the fact that these cells eliminate their secretion in a holocrine fashion. The morphological aspects suggest that the cells lining the granular ducts in albino Wistar rat mandibular gland present merocrine and apocrine secretory mechanisms. Besides, they also present holocrine secretory mechanism, the first time reported in this species.

## Introduction

The saliva in the oral cavity is the sum of secretion arrived here from salivary glands. Once it reaches the collecting ducts, the composition of the saliva secreted by the acini is modified (Bowen, 2002). Usually, salivary glands contain three types of ducts: intercalary (ducts of Boll), striated and excretory (principal) (Edgar, 2004). Among them, the striated ducts are majoritary (Fejerskov and Kidd, 2003). In the mandibular gland of rat, there is another type of duct present, lined by cells containing membrane-bound granules in the apical pole. Due to this fact, they are termed as granular ducts (Kanno, 2004), and in this species, they are majoritary, to the detriment of the striated ones. Some authors studied the development of granular ducts from rat mandibular gland, observing a heterogeneity of the intracytoplasmatic granules at the age of 6 weeks. They state that the presence of heterogeneous granules could indicate a functional diversity of the cells (Srinivasan and Chang, 2005). In rats, the area in close vicinity to the intercalary ducts differentiates at puberty, when it presents acidophilic granulations (Quissel and Redman, 1979). As a matter of fact, this is the moment when the ducts become granular. Reabsorption and

Received 14 September 2017; accepted 29 May 2017.
**doi:** 10.ANN/RSCB-2017-0016:RSCB

secretion processes take place in these ducts. In adult rodents, granular ducts are lined by the following type of cells: granular, dark granular, pillar and transition (Mori et al., 1992). The secretion mechanism of these cells is different, depending on the secreted substance. After alpha-adrenergic stimulation, the granular cells release the following substances: biologically active peptides, processing enzymes, epidermal growth factor, nerve growth factor, kallikrein, renin, erythropoietin, cysteine, glucagon, somatostatin etc., and the pillar cells: S-100 protein and neuron specific enolase (Mori et al., 1992; Edgar, 2004; Martini, 2006). During degranulation of the cells lining the granular ducts in rat, the glycogen represents an important energy source (Garrett et al., 1994). The aim of this study was to assess the secretory mechanisms present in cells lining the granular ducts in the mandibular glands in albino Wistar rat.

## Materials and methods

The study was approved by the Bioethics Committee of University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca and the biological material was represented by 5 male albino Wistar rats. We sacrificed the animals by anesthetizing them with isoflurane (Aerrane isoflurane; Baxter S.A.) for a prolonged period of time. We harvested the mandibular glands and histologically processed them. The samples were firstly fixed in 10% buffered formalin, for one week. The fixation solution was renewed twice during the fixation period, in order to make sure all structures are fixed accordingly. The next step comprised dehydration with ethanol, in increasing concentrations (500, 700 and absolute). The clarification was achieved using n-butanol and the samples were subsequently embedded in paraffin. After shaping paraffin blocks, containing the tissue inside, we sectioned the samples at a 5 µm thickness, with the aid of Leica rotary microtome. The slides containing the tissue were stained with Goldner's trichrome staining procedure and examined under an Olympus BX41 microscope, equipped with an Olympus E-330 digital camera. The images were processed with Adobe Photoshop CS2 software.

## Results and discussions

We identified cells presenting bulges in the apical pole with a tendency to strangle and detach as vesicles (Fig. 1-2).

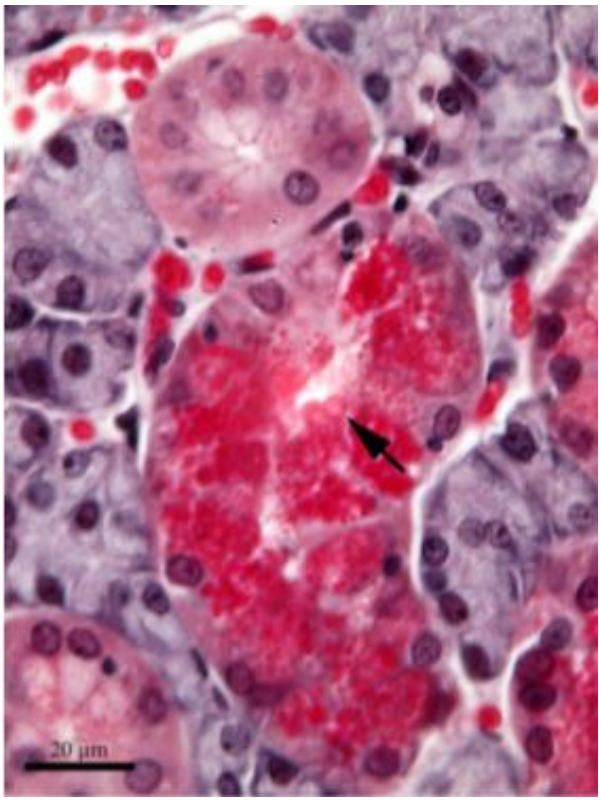


**Fig 1.** Mandibular gland in Wistar rat - apocrine secretory mechanism (black arrow)

Annals of R.S.C.B., Vol. XXI, Issue 3, 2017, pp. 7 – 12
Received 14 September 2017; accepted 29 May 2017.
**doi:** 10.ANN/RSCB-2017-0016:RSCB

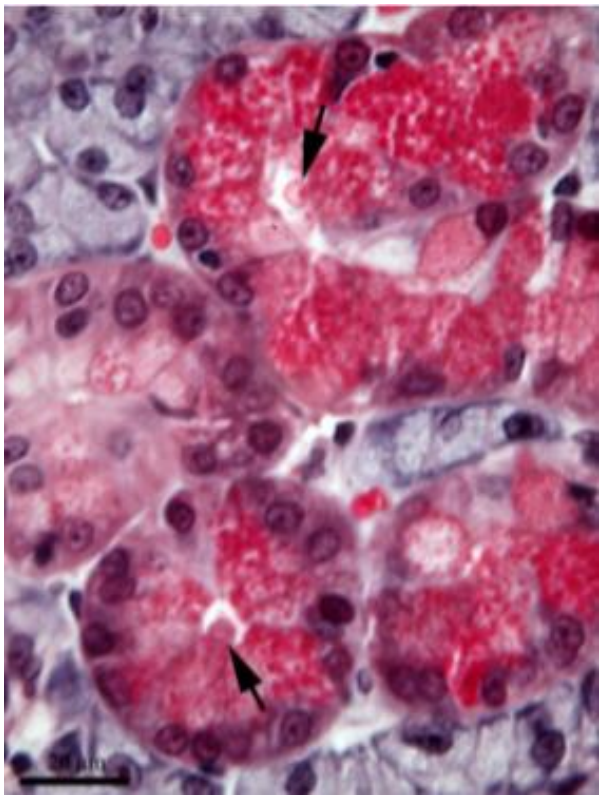

**Fig. 2.** Mandibular gland in Wistar rat - apocrine secretory mechanism (black arrow)

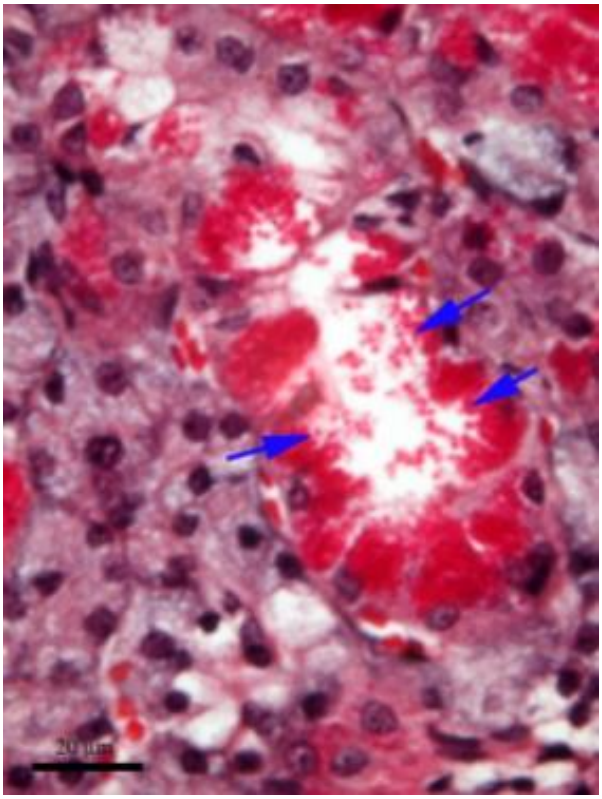

**Fig 3.** Mandibular gland in Wistar rat - holocrine secretory mechanism (blue arrows).

These aspects suggest the fact that some of the cells lining the granular ducts in the mandibular gland in rat eliminate the secretion product in an apocrine fashion. The number of these cells is not big, which shows that the apocrine secretory mechanism is present, but only in a small number of cells.

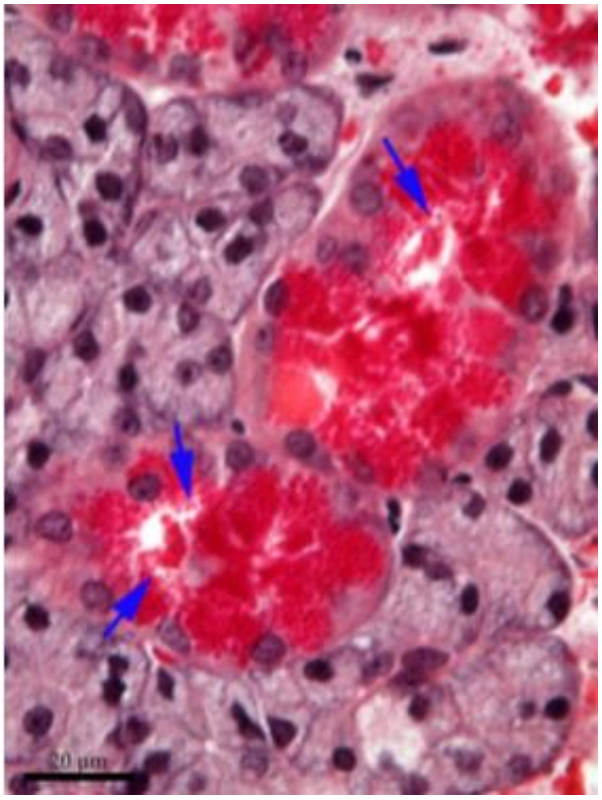

**Fig. 4.** Mandibular gland in Wistar rat - holocrine secretory mechanism (blue arrows).

**doi:** 10.ANN/RSCB-2017-0016:RSCB
Received 14 September 2017; accepted 29 May 2017.

Other cell present discontinuities in the apical pole and a tendency to eliminate all the cytoplasmic components (whole granules or granule fragments), which can be highlighted in the ductal lumen (Fig. 3-4). The aspect suggests the fact that these cells eliminate their secretion in a holocrine fashion. These cells are more numerous than the one presenting an apocrine secretory mechanism, but not in comparison to the ones which do not present discontinuities of the apical pole and seem like to eliminate their secretory products by merocrine mechanism.

## Discussion

The noticed aspects suggest that most of the cells from the granular ducts in the mandibular gland in rat eliminate their secretory products through merocrine secretory mechanism, other through apocrine secretory mechanism, aspects also observed by other researchers. Besides these mechanisms, we also identified cells eliminating their secretion through a holocrine secretory mechanism, but we state that they are in relatively small numbers. Mese and Matsuo (2007) mention that saliva formation has two phases: formation and modification. The initial stage implies the primary secretion of acini and the modification stage takes place in the ducts. The authors write that stimulation of the sympathetic nerve or β-adrenergic stimulation cause exocytosis and the secretory process unreels by fusion of granule membrane with the luminal one of the secretory cell, followed by the rupture of the fused membranes (Mese and Matsuo, 2007). Other authors also mention the presence of fused granules in the cells lining the granular ducts, with their subsequent elimination in the lumen (HazenMartin and Simson, 1986). Granular ducts possess an intense secretory activity, suggested by the shape (contort) and length of the ducts and the large number of granules present in the cytoplasm of the cells lining them. Among the substances secreted by the granular duct cells, we mention bioactive polypeptides, hormones, growth factors. The authors write that there is no doubt that these substances are

produced by the granular duct cells and are secreted into the lumen in an exocrine fashion (Amano et al., 2012). The fact that the cells secrete different substances is confirmed by the polymorphism, in both size and tinctorial affinity, of the granulations. The secretory granules from the granular ducts in rat mandibular gland form after the fusion of small granules, a fairly slow process (Thomopoulos et al., 2002). Thus, the final secretion product of the gland will be formed out by the glandular cells in the acini, mixed with the secretion from the granular duct cells. Interestingly, sometimes the secretion of the granular ductal cells seems to exceed the one from the acinar cells. The apocrine secretory mechanism was signaled by one author, years ago, mentioning the fact that the apical part of the cells lining the striated ducts develops a process, a bulge which grows in size and finally detaches and ends up in the lumen, forming the secretory product of these ducts (Takano, 1976). He states that the cells from the striated ducts can be divided into 3 areas. The first one is the apical, immediately next to the lumen, which does not contain secretory granules. The second area contains abundant granules and the third contains the organelles. The first area, or separation zone, is the one detaching from the main body (the third area) (Takano, 1976). Other authors mention that they observed the elimination of secretory granules in the apical pole of the granular duct cells through exocytosis (Qwarnstrom and Hand, 1983). It seems that the secretory activity is under nervous control (Quissel and Redman, 1979), and the cells lining the granular ducts from rat mandibular gland present different responses depending on the stimulation of the autonomic nerves (Andreson et al., 1995). Nevertheless, it seems that release of the secretion products takes place spontaneously, through exocytosis, in the case of aggressive behavior in mice (Aloe et al., 1986). After intermittent sympathetic stimulation, the changes appeared in the cells are not synchronous, not even in the neighboring cells. Yet, once the cell begins the secretory process, it unreels similarly for all cells

**doi:** 10.ANN/RSCB-2017-0016:RSCB

(Thomopoulos et al., 2000). Other authors mention that the cells from granular ducts in rat respond differently according to the autonomic nerve stimulation (Anderson et al., 1995). It seems that not all cells respond to the stimulation, but in the ones which respond, the intracytoplasmatic granules align towards the apical membrane, triggering classic exocytosis phenomena. The granules inside the cell fuse, form larger aggregates, with an irregular shape. The secretion takes place by discharging these aggregates in the lumen of the granular duct (Thomopoulos et al., 2000). Tamarin and Shreebny (1965) mentioned that this ballooning of the cells lining the granular ducts from the mandibular gland in rat is influenced by tissue fixation for the subsequent histological processing. They state that the dilatation of the apical pole emerges when a hypo-osmolar fixation solution is used. However, they state that small blebs appear in this case. Tandler et al. (2001) believe that the emergence of these dilatations are due to the fixation process, stating that they appear in glands fixated by vascular perfusion. Others state that the aspect is normal because, after X ray exposure, the cells lose their ability to form blebs (Messelt and Dahl, 1983). Other authors studied the mandibular glands (in harbor seal and rat) and concluded that the dilatation of the apical pole is a form of apocrine secretion (of the apocrine secretory mechanism) (Messelt, 1982; Messelt and Dahl, 1983). In scientific literature, the mentioned secretion mechanisms are represented by typical exocytosis and apocrine secretory mechanism and Thomopoulos et al. (1996) also mention the compound exocytosis, with aggregate formation. We observed the merocrine and apocrine secretory mechanisms, but beside them, we also signal the presence of holocrine secretory mechanism in a number of cells lining the granular ducts in Wistar rat mandibular gland.

## Conclusion

The morphological aspects noticed suggest that the majority of cells in the albino Wistar rat mandibular gland eliminate their secretion through a merocrine secretory mechanism and some through an apocrine secretory mechanism. Besides these mechanisms, we also identified a small number of cells which eliminate their secretory products through a holocrine secretory mechanism. This is the first report on the presence of holocrine secretory mechanism in albino Wistar rats.

## References

Aloe, L.; Alleva, E.; Bohm, A.; Levi-Montalcini, R.: Aggresive behavior induces release of nerve growth factor from mouse salivary gland into the bloodstream. Proc Nat Acad Sci USA, 83, 61846187, 1986

Amano, O.; Mizobe, K.; Bando, Y.; Sakiyama, K.: Anatomy and Histology of Rodent and Human Major Salivary Glands. Acta Histochem Cytochem, 45, 5, 241–250, 2012

Anderson, C.L.; Garrett, J.R.; Zhang, X.; Proctor, G.B.; Shori D.K.: Differential secretion of proteins by rat submandibular acini and granular ducts on graded autonomic nerve stimulations. Journal of Physiology, 4495, 2, 503-511, 1995 Bowen, R.: Salivary Glands and Saliva. Colorado State University. Retrieved Oct 13, 2009 from http://www.vivo.colostate.edu/hbooks/pathphys/digestion/pregastric/salivary.html, 2002

Edgar, W.M., Chapter 2. Mechanisms of Salivary Secretion (Smith P.M.), in: Saliva and Oral Health 3rd Edition, 2004. Publisher: British Dental Journal, edited by W.M. Edgar, London, United Kingdom

Fejerskov, O.; Kidd, E., Dental Caries: The Disease and its Clinical Management (1st ed.), 2003. Oxford: Wiley-Blackwell.

Garrett, J.R.; Thomopoulos, G.N.; Zhang, X.S.; Hartley, R.: The fate of glycogen in granular tubule cells of rat submandibular glands during secretory events. Arch Oral Biol., 39, 5, 449-452, 1994

Hazen-Martin, D.D.; Simson, J.A.V.: Ultrastructure of the secretory response of male mouse submandibular gland granular tubules. The Anatomical Record, 214, 3, 253-265, 1986

Kanno, T.: Compound exocytosis of secretory granules containing salivary chromogranin A in granular duct cells in rat submandibular gland: the last study in collaboration with the late Professor Noboru Yanaihara at Yanaihara Institute. Regulatory Peptides. 123, 1-3, 3-7, 2004

Martini, H.F., Fundaments of Anatomy & Physiology (7th ed.), 2006. Benjamin Cummings. Mese, H.; Matsuo, R.: Salivary secretion, taste and hyposalivation. J Oral Rehabil, 34, 10, 711-723, 2007

**doi:** 10.ANN/RSCB-2017-0016:RSCB

Messelt, E.B.; Dahl, E.: Influence of X-ray irradiation on the ultrastructure of rat submandibular gland striated-duct cells. Acta Odont Scand, 41, 277–282, 1983

Messelt, E.B.: Ultrastructural studies on the bleb formation in seal and rat submandibular gland striated ducts. Acta Odont Scand, 40, 25–33, 1982

Mori, M.; Takai, Y.; Kunikata, M.: Review: biologically active peptides in the submandibular glands—role of the granular tubules. Acta Histochem Cytochem, 25, 325–341, 1992 Quissel,

D.O.; Redman, R.S.: Functional characteristics of dispersed rat submandibular cells, Proc. Natl. Acad. Sci. USA, 76, 6, 2789-2793, 1979 Qwarnstrom,

E.E.; Hand A.R.: A granular cell at the acinar-intercalated duct junction of the rat submandibular gland. The Anatomical Record, 206, 2, 181-187, 1983

Srinivasan, R.; Chang, W.W.L. The development of the granular convoluted duct in the rat submandibular gland. The Anatomical Record, 182, 1, 29-39, 2005

Takano, K.: Shift of secretory granules in the striated duct cell of the rat submandibular gland. Journal of Electron Microscopy, 25, 3, 151-154, 1976

Tamarin, A.; Sreebny, L.M.: The rat submaxillary salivary gland. A correlative study by light and electron microscopy. Journal of Morphology, 117, 3, 295-352, 1965

Tandler, B.; Gresik, E. W.; Nagato, T.; Phillips, C. J.: Secretion by striated ducts of mammalian major salivary glands: review from an ultrastructural, functional, and evolutionary perspective. Anat. Rec., 264, 121–145, 2001

Thomopoulos, G.N.; Garrett, J.R.; Proctor G.B.: Ultrastructural histochemical studies of secretory processes in rat submandibular granular tubules during intermittent sympathetic nerve stimulation. Eur J Morphol, 38, 2, 69-79, 2000

Thomopoulos, G.N.; Garrett, J.R.; Proctor, G.B.: Ultrastructural histochemical studies of secretory granule replenishment in rat submandibular granular tubules after cyclocytidine-induced secretion. J Submicrosc Cytol Pathol, 34, 3, 279-289, 2002

Thomopoulos, G.N.; Garrett, J.R.; Proctor, G.B.; Hartley R.; Zhang X.S.: Exocytosis from rat submandibular granular tubules during cyclocytidine stimulation shows unusual features, including changes in the granule membrane. Microsc Res Tech, 35, 5, 365-376, 1996