Asta Tvarijonaviciute
Silvia Martínez-Subiela
Pia López-Jornet · Elsa Lamy *Editors*

Saliva in Health and Disease

The Present and Future of a Unique Sample for Diagnosis

Springer

Saliva in Health and Disease

Asta Tvarijonaviciute
Silvia Martínez-Subiela • Pia López-Jornet
Elsa Lamy
Editors

Saliva in Health and Disease

The Present and Future of a Unique Sample for Diagnosis

Springer

Editors
Asta Tvarijonaviciute
Interdisciplinary Laboratory of Clinical Analysis (Interlab-UMU), Regional Campus of International Excellence Campus Mare Nostrum
University of Murcia
Murcia, Spain

Pia López-Jornet
Department of Oral Medicine, Faculty of Medicine and Dentistry, Regional Campus of International Excellence Campus Mare Nostrum
University of Murcia
Murcia, Spain

Silvia Martínez-Subiela
Interlab-UMU
University of Murcia
Murcia, Spain

Elsa Lamy
MED - Mediterranean Institute for Agriculture Environment and Development, IIFA - Instituto de Investigação e Formação Avançada
University of Évora
Évora, Portugal

ISBN 978-3-030-37680-2 ISBN 978-3-030-37681-9 (eBook)
https://doi.org/10.1007/978-3-030-37681-9

This Springer imprint is published by the registered company Springer Nature Switzerland AG.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

In the past, saliva was considered just as a component of the digestive process with a main role of initiating the breakdown of lipids and starches. However, during the last years, the view of saliva in biological sciences has changed dramatically; it was shown to contain a variety of molecular and bacterial compounds that can change in local and systemic pathologies. Furthermore, a number of proteins that are present in saliva are absent in plasma, representing an opportunity to identify new molecular biomarkers of different conditions such as disease diagnosis and treatment monitoring. Nevertheless, currently, blood is the sample most frequently used for health assessment in human and veterinary medicine. However, blood sampling is physically intrusive and technically demanding. In contrast, the use of saliva supposes a reduction of stress associated with the sampling due to its noninvasive nature and a decrease in costs because there is no need for a specialized personal for the sampling. In the particular case of elderly persons, children and animals, the samplings could be performed at home without the need to go to the clinic or hospital to avoid stressful situations. However, the use of saliva in routine practice is still limited. This is mainly due to *the lack of knowledge*. Therefore, in order to explore the diagnostic potential of this biofluid, existing literature related to salivary biomarkers in both humans and animals were gathered together, giving place to the current form of this book. The chapters of it could be grouped into three: (1) *generalities*, which describe anatomy and physiology of salivary glands (Chap. 1), saliva and its biomarkers role in ingestive behaviour (Chap. 2), pros and cons of saliva usage as a diagnostic biofluid (Chap. 3), the methodologies for salivary biomarker identification and validation (Chap. 4) and main challenges restricting the use of saliva in a clinical settings from the scientific point of view and the possible ways to solve them (Chap. 15), (2) *oral and systemic pathologies* (Chaps. 5–12) and (3) *physical and psychological stress and welfare* studies (Chaps. 13 and 14).

It is important to highlight that each of the chapters was integrated not only by the knowledge existing in human medicine but also for animals. This was performed in agreement with One Health approach, stating that integration of knowledge leads to higher quality or larger quantity of relevant information, leading to more economically efficient research that drives to more accurate conclusions.

Murcia, Spain — Asta Tvarijonaviciute, Silvia Martínez-Subiela, Pia López-Jornet

Evora, Portugal — Elsa Lamy

Acknowledgements

We, the editors, would like to thank all authors who agreed to participate in this project, making the production of this book possible. Also, we are grateful to Dr. Gonzalo Cordova for his help in every moment and for his valuable advices.

This book would not be possible without the financial support given by the Seneca Foundation of Murcia Region through projects 20649/JLI/18 and 19894/GERM/15, the Ministry of Economy and Competitiveness of Spain through the postdoctoral programme "Ramon y Cajal" and the project AGL2016-79096-R, and COST (European Cooperation in Science and Technology) Action 16215 the European network for the promotion of portable, affordable and simple analytical platforms (PortASAP). Thanks are also due to the FCT-Portuguese Science Foundation under the project UID/AGR/00115/2013 and the research contract CEEIND to Elsa Lamy.

Contents

Part I
Saliva Basics

Chapter 1
Salivary Glands' Anatomy and Physiology

María D. Contreras-Aguilar and Francisco Gómez-García

Objectives
This contribution aims to show the anatomical and physiological characteristics of the salivary glands as entity for the production of saliva, and to present the composition of the saliva fluid as a protective medium for the mouth, the start of digestion and as diagnostic medium. All this from a comparative point of view between humans and animals.

1.1 Salivary Glands' Anatomy and Histology

The salivary glands are part of the digestive tract (Nater and Rohleder 2009), and their major physiological function is to secrete saliva into the oral cavity, which is essential for the lubrication, digestion, immunity, and overall maintenance of homeostasis within the body.

M. D. Contreras-Aguilar (✉)
Interdisciplinary Laboratory of Clinical Analysis (Interlab-UMU), Veterinary School, Campus of Excellence Mare Nostrum, University of Murcia, Murcia, Spain
e-mail: mariadolores.contreras@um.es

F. Gómez-García
Department of Dermatology, Stomatology, Radiology and Physic Medicine. Faculty of Medicine. Campus of Excellence Mare Nostrum, University of Murcia, Hospital Universitario Morales Meseguer, Murcia, Spain
e-mail: fjgomez@um.es

A. Tvarijonaviciute et al. (eds.), *Saliva in Health and Disease*,
https://doi.org/10.1007/978-3-030-37681-9_1

1.1.1 Types of Salivary Glands

Salivary glands are classified as major and minor salivary glands. The terms *major* and *minor* are associated to the anatomic size of the glands and to the volume of saliva produced (Edgar 1990).

The major salivary glands are bilateral paired glands and include parotid (PG), which are located opposite the maxillary first molars, and submandibular (SMG) and sublingual glands (SLG), which are found in the floor of the mouth (Ferreira and Hoffman 2013; Humphrey and Williamson 2001). Although they are distributed in a horseshoe shape from the midline to the external auditory canal, bordering the jaw in human (Velayos and Santana 2007); in domestic animals, not all are equally developed; neither its topographic localization is the same nor the salivary ducts arranged identically in all of them. In horses and swine, as well as in human, PG is the most developed, with an extension from the base of the ear to zone of external jugular vein conformation. The SMG in cows is the most developed and it is the one that brings more saliva secretion. In horses and humans, the SMG is divided in its cervical extreme placed in the cervico-facial transit, and in its rostral extreme placed in the inter-mandibular space. In human, SMG is the second largest salivary gland, extended from mandibular angle to the hyoid bone. The SLG in domestic animals are divided in polystomatic and monostomatic SLG, although only the polystomatic presented in the horse. In dogs, a differentiated gland appears, named Zigomatic gland, which derives from the minor dorsal buccal glands in relation with the pterygopalatine fossa (Vázquez-Autón et al. 2002).

The minor salivary glands are distributed in groups of hundreds in the upper digestive tract mucosa, eg, in the lower lip, tongue, palate, cheeks, and pharynx (Roth and Calmes 1981). However, in domestic animal, there are other minor salivary glands topographed in relation to the buccinator muscle, the dorsal and ventral buccal glands, although there are also intermediate buccal glands in ruminants (Sandoval 1998; Vázquez-Autón et al. 2002).

1.1.2 Histology

Salivary glands are exocrine and are comprised of different types of cells: acinar cells, various duct system cells, and myoepithelial cells (Humphrey and Williamson 2001) (Fig. 1.1). The glandular histophysiological unit is called sialone and comprises the acinar cells as a secretory piece similar to a cluster of grapes (adenomere), and the ductal portions performed by the duct system cells inside the lobules (intralobulal ducts). However, in the minor salivary glands, subdivision into lobules is not always complete and they have short, individual excretory ducts (Chimenos Kustner et al. 2012). Acinar cells secret firstly the saliva into the ductal lumen, which could be classified as serous, mucous, or mixed (Fig. 1.1). In fact, the quality of saliva

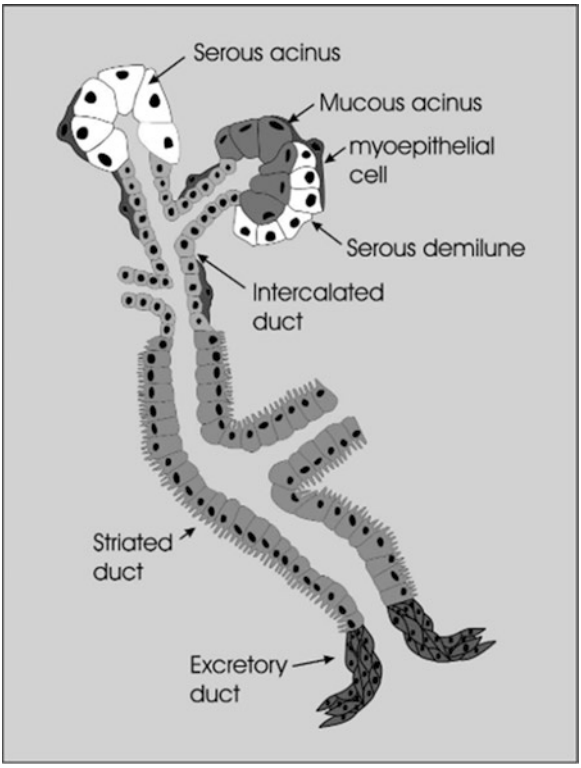

Fig. 1.1 Salivary glandular tissue ducts. (Figure taken from Edgar et al. 2012)

content varies according the histology of each salivary gland (Edgar 1990): the PG produces mainly serous secretions, minor glands mainly mucous secretions, and SLG and SMG produce mixed serous and mucous secretions. Duct system in the major salivary glands is complex and comprises intercalated, striated, and excretory ducts. The first two are shaped by a simple epithelium and are located inside the lobules, so they are called intralobular ducts. While intercalated cells are not involved in the modification of electrolytes, striated cells are responsible for electrolyte regulation. The final ducts called excretory or collector ducts, are shaped by a bilaminar epithelium with a large light surrounded by connective tissue and contribute in the modification of electrolytes as well. They are the last part of the duct network and lead saliva to the oral cavity (Fig. 1.1). With respect to the last cellular type, the myoepithelial cells, they are arranged intimately around acinar cells, being part of the basal layer. Its function is to contract to constrict the acini in order to make secreting or "squeezing out" accumulating fluid. This secreting process is purely neural (Chimenos Kustner et al. 2012; Edgar 1990; Roth and Calmes 1981). Finally, a large amount of adipocytes may be found in the stroma, mainly in PG from human, which with the age a large part of the functional parenchyma might be replaced by adipose tissue (Velayos and Santana 2007).

1.1.3 The Excretory Ducts in Major Salivary Glands

The excretory ducts from the major salivary glands are formed when several interlobular excretory ducts join and lead the saliva to the oral cavity. Like the salivary glands, these excretory ducts are bilateral paired.

The parotid duct in human, called *Stensen duct*, emerges rostral to the gland, and runs forward along the lateral side of the masseter muscle until crossing the buccal fat pad (an encapsulated fat mass in the cheek, presented in humans and porcine) (Niada et al. 2013), to then taking a steep turn at the border of the masseter and to pass through the buccinador muscle to open into the vestibule of the mouth on the parotid papilla (small bumps), which is lied across the second superior molar tooth (Velayos and Santana 2007). However, its layout is different with respect to domestic species. For instance, in horses, the parotid duct begins also rostral to the gland but runs ventromedial to the mandible along the pterygoid medial muscle, and then it turns dorsally to pass the lateral mandible across the mandibular notch together the facial artery and vein, running after between the facial vein and masseter muscle. After pass through the buccinador muscle, it leads on the parotid papilla lied into the vestibule of the mouth across the third or fourth maxillary premolar. In the case of cattle or small ruminants, this duct runs along the ventral surface of the masseter muscle; however, with respect to the dog, the parotid duct runs similar to human along the lateral side of the masseter muscle (Sandoval 1998).

With respect the mandibular duct, or in human the *Wharton duct*, its trajectory is on the bucal floor between the mylohyoid, hyoglosus and genioglosus muscles to run rostral to the lingual frenulum to lead in the sublingual caruncle (Sandoval 1998; Vázquez-Autón et al. 2002; Velayos and Santana 2007).

The SLG in human has several excretory ducts called *Walther's ducts* that lead in the sublingual caruncles. In the monostomatic SLG from domestic animals such as cattle or dogs, there is a major sublingual duct, which is similar to the largest *Walther's ducts* in human, called *Bartholino duct*; that runs alongside the mandibular duct discharging their excretion into the sublingual caruncles. However, the polystomatic SLG discharge salivary secretions through severals minor sublingual ducts located in the sublingual recess of the mucosal fold (Sandoval 1998; Vázquez-Autón et al. 2002; Velayos and Santana 2007).

Finally, and particularly in the dog, the zygomatic duct from the zygomatic gland leads into the vestibule of the mouth on the zygomatic papilla, caudal to the parotid papilla (Sandoval 1998).

1.2 Saliva Secretion

1.2.1 Regulation

The functional innervation of the human salivary glands is known from studies performed with animal models (Ferreira and Hoffman 2013). Acinar cells and their associated myoepithelial cells are both innervated by both the sympathetic and the parasympathetic branches of the autonomic nervous system (ANS), and there is no antagonism between the two branches (Emmelin 1987). Parasympathetic nerve impulses produce high-flow, low-protein saliva, whereas sympathetic impulses produce low-flow, high-protein saliva (Fig. 1.2). However, these are not absolutes. Parasympathetic stimuli can increase the exocytosis from salivary cells for releasing proteins in saliva, but seem particularly important for the secretion of mucins on mucous gland secretion; and adrenergic stimuli can invoke some salivary flow but not as part of the salivary reflex (Carpenter 2013; Proctor and Carpenter 2007). As result, the resting flow rate observed in human sampled by passive drooling ranges from 0.25 to 0.90 mL/min with a mean of approximately 0.4 mL/min, increasing when a sensory stimuli (visual, olfactory or taste) or parasympathetic activity happens (Beltzer et al. 2010; Chimenos Kustner et al. 2012; Thie et al. 2002). Although other factors may vary the resting salivary secretions, such as the age, number of teeth, sex, body weight the circadian rhythmic, or some medications; as well as some diseases such as hepatic disease, malnutrition, depression, neurological diseases, diabetes, chronic painful disorders, among other (Thie et al. 2002).

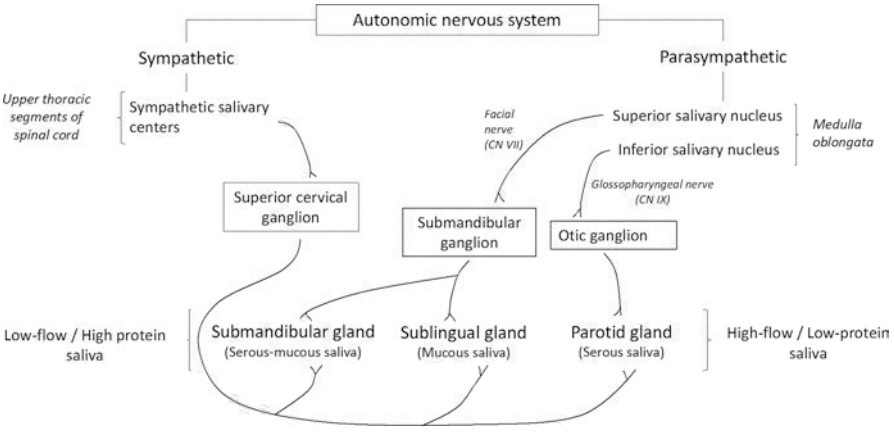

Fig. 1.2 Scheme on the regulation of salivary flow and protein production

1.2.1.1 Salivary Gland Innervation Routes

Preganglionic parasympathetic innervation of the PG comes from fibers of the IX cranial nerve (glossopharyngeal), which are conducted from the medullary region of the brainstem in the inferior salivatory nucleus (ISN) to its synapse in the otic ganglion (Fig. 1.2). Subsequently, the postganglionic fibers of the otic ganglion reach the PG, providing parasympathetic innervation for the secretion of serous-watery saliva. On the other hand, the innervation of both the SMG and the SLG is due to parasympathetic preganglionic fibers belonging to the facial nerve (or cranial nerve VII). These come from the superior salivary nucleus (SSN) to reach the submandibular ganglion, where short postsynaptic fibers leave the ganglion to innervate the SMG and SLG, which secrete serous-mucous and mucous saliva, respectively (Davis et al. 1956).

On the other hand, the sympathetic branches come from the sympathetic salivary centers located in the upper thoracic segments of the spinal cord. The paravertebral sympathetic trunk carries the ascending preganglionic fibers from the thoracic ganglion to synapse at the superior cervical ganglion (SCG). Postganglionic sympathetic fibers exit the SCG and the sympathetic fibers give off branches to reach all three pairs of major salivary glands through the external carotid artery plexus and its branches, as the facial artery (Kahle et al. 2010). These branches take charge of regulating peripheral blood flow, salivary secretion, and local inflammatory and immune mediators (Emilio Savastano et al. 2010; Mathison et al. 1994).

1.2.1.2 Neurotransmitters Released by Autonomic Nerves Innervating Salivary Glands

The major types of neurotransmitters released by the autonomic nervous fibers that innervate the salivary glands are the acetylcholine (ACh) and noradrenaline (NA), which provides cholinergic and adrenergic signaling responses, respectively (Fig. 1.2). ACh is mainly the neurotransmitter between preganglionic and postganglionic neurons in both the sympathetic and parasympathetic fibers, and between postganglionic parasympathetic neurons and salivary glands. Meanwhile, between postganglionic sympathetic neurons and effector salivary glands, NA is the major neurotransmitter. Calcitonin gene-related peptide (CGRP), Neuropeptide Y (NPY), neurokinin A (NKA), neuronal nitric oxide synthase (nNOS), pituitary adenylate cyclase activating peptide (PACAP), substance P (SP) and vasoactive intestinal peptide (VIP) are other non-adrenergic, non-cholinergic transmitters released from either parasympathetic, sympathetic or both autonomic nerves in salivary glands. All of them are neuropeptides with effects on the blood vessels and on the salivary cells which can modify the protein and/or fluid secretion (Ekström 1999; Ekström et al. 1989). For example, together with Ach, VIP is capable to cause vasodilation in the SMG to increase blood flow and salivary secretion (Lundberg et al. 1981). Or SP and/or CGRP can be found in sensory nerve fibers from the sympathetic and parasympathetic nerve bundles which target ducts and blood vessels in rat SMGs (Kobashi et al. 2005).

1.2.2 Pathways of Saliva Secretion

It must be emphasized that the effects of parasympathetic and sympathetic nerve impulses on protein and fluid secretion from salivary glands can differ between glands in the same species and between the same gland in different species (Proctor and Carpenter 2007). Acinar cells are responsible for the secretion of the primary fluid and of most of the proteins found in saliva (>85%), although duct cells secrete numerous proteins with important biological activities, e.g., nerve growth factor, epidermal growth factor, immunoglobulin (Ig) A, and kallikrein (Melvin et al. 2005). The different elements that compound the gland-derived salivary constituents are transported from the salivary glands' cells by:

- *Process of exocytosis* ➔ Most of the proteins secreted by salivary glands are derived from protein storage granules in acinar cells, which are then released by a process of exocytosis (Castle et al. 1975; Segawa and Yamashina 1998). Those proteins have to be firstly synthetized and packaging (Edgar et al. 2012). The exocytosis starts when neurotransmitters, mainly by a sympathetic stimulation, but also by a parasympathetic one; exert their activity at the cell membrane (Asking and Gjorstrup 1987; Nater and Rohleder 2009). They bind to specific receptor proteins on the basolateral membrane, causing a degranulation of the storage granules. Na^+ from sympathetic neurons binds to both α - and β-adrenergic receptors on the acinar cell. α1-receptor activation is linked to elevation of intracellular Ca^{2+}, which results in large-scale fluid and electrolyte transport, and modest exocytosis of stored protein; while β1-receptor activation causes elevation of intracellular cyclic adenosine monophosphate (cAMP) followed by activation of protein kinase A and phosphorylation of endogenous proteins leading the fusion of secretory granules with the apical membrane of cells (Baum 1993; Castle and Castle 1998). In addition, neuropeptide VIP via parasympathetic activation can also acts on the exocytosis process through cAMP; and cholinergic stimuli or substance P can give rise to the release of protein by a coupling mechanism involving elevated intracellular Ca^{2+} and activation of protein kinase C (Ekström et al. 1989; Moller et al. 1996). For example, this is the way of excretion of the salivary alpha-amylase (sAA).
- *Vesicular protein transport (endocytosis)* ➔ Other way to protein secretion is the vesicular protein transport, as occurs with the secretory IgA. This is actively carried across acinar and ductal cells via a transporter protein, that in the case of the IgA in called the polymeric immunoglobulin receptor (pIgR). Although this process can be up regulated by neural activity due to a parasympathetic and sympathetic activation (Carpenter et al. 1998; Proctor and Carpenter 2002), it does not involve storage of protein within cells. In the case of IgA, which is made by plasma cells located in the connective tissues' gland, binds to the pIgR on acinar and ductal cells on the basolateral surface and it is endocytosed into the cell. Then, the vesicle is transported across the cell to the apical membrane, where the membrane receptor is cleaved to release secretory IgA (the secretory component is the cleaved part of the pIgR). The pIgR is specific to IgA, and so even though

there are equal numbers of IgG-, IgA-, and IgM-producing plasma cells within the gland (Mega et al. 1992), IgA becomes the single-most- abundant antibody in saliva because it is preferentially bound by the pIgR (Brandtzaeg 1998). IgG and IgM probably entry into the oral cavity via crevicular fluid (Eliaz Kaufman and Lamster 2000).

- Intracellular diffusion ➔ This is the case of cortisol, estriol and testosterone in saliva, which are transported passively (transcellular) from plasma as nonprotein-bound (unbound) fraction to saliva by diffusing through the cells of the salivary glands due to its solubility in the lipid-rich cell membranes (Vining et al. 1983). As result, its concentration in saliva does not depend on the rate of saliva production (Büttler et al. 2018; Vining et al. 1983). In the case of cortisol, it is only presented in saliva as free cortisol (unbound fraction) (Perogamvros et al. 2011).
- *Ultrafiltration* ➔ Ultrafiltration in salivary glands is the passive transport of compounds from plasma to saliva via the tight junctions between the acinar cells (paracellular) due to a positive hydrostatic pressure. It is only allowed for compounds with a relative molecular mass cutoff of approximately 100–300. This way of transport occurs in lipid-insoluble conjugated steroids, such as thyroxin or choriogonadotropin (Vining et al. 1983). This rout is by which water also crosses the cells of the salivary glands when a transepithelial osmotic gradient or higher hydrostatic pressure happen (Young et al. 1987). On this last case, the parasympathetic and sympathetic exercise on parenchymal cells short-term regulates the salivary gland blood flow increasing the hydrostatic pressure after an activation. E.g, the parasympathetic stimulus produce vasodilation within an integral salivary reflex (Anderson et al. 2006; Mizuta et al. 2000); while the sympathetic-mediated vasoconstriction, depends on a separate vasomotor control independent of the salivary reflex (Emmelin and Engstrom 1960). In addition, the myoepithelial cells, which surround glandular acini, are contracted by the stimulus of parasympathetic and sympathetic fibers (Tamarin 1966).
- *Water channels or aquaporins transport* ➔ Aquaporins are water channels that allow the transepithelial water movement. There are five aquaporins expressed in salivary glands (Aqp1, Aqp3, Aqp4, Aqp5, and Aqp8). However, Aqp5, which is highly expressed in the apical membranes of salivary acinar cells (Funaki et al. 1998; Matsuzaki et al. 1999), appears to be the only aquaporin to play a major role in salivation (Gresz et al. 2001; Melvin et al. 2005).
- *Active transport of ions by channels* ➔ Fluid secretion depends critically on the movement of Cl^-, K^+ and HCO^{-3} ions through Ca^{2+}-activated channels or cotransporters in the luminal membrane (transcellular) (Melvin et al. 2005). The release of ACh from parasympathetic nerves and its interaction with muscarinic cholinergic receptors (mAChRs) principally regulates fluid secretion on the salivary glands. Stimulation via mAChR receptors is coupled to secretion of inositol triphosphate (IP3) and diacylglycerol. The interaction of IP3 with its specific receptor on the endoplasmic reticulum causes release of stored Ca^{2+} (Baum and Wellner 1999). Rises in intracellular Ca^{2+} open, for example, apical membrane Cl^- channels and basolateral membrane K^+ channels in acinar cells. When Cl^- goes out through the acinar apical membrane and K^+ enters the interstitial fluid is

created a trans-epithelial potential difference. This electrical potential difference allows the passive exchange of cations across the tight intercellular junctions and the luminal accumulation of ions. This generates a transepithelial osmotic gradient that enables the movement of water via paracellular (ultrafiltration) or transcellular (water channels or aquaporins transport) to create the primary fluid secretion, which ionic composition in the interstitial fluid bathing the basolateral aspect of the acinar cells. In addition, the Na^+/HCO^{-3} cotransporter activity plays a potential role in salivary secretion due to the electrogenic HCO^{-3} conductance generated. However, it appears to be both gland- and species-specific (Kim et al. 2003; Luo et al. 2001; Melvin et al. 2005).

1.3 Saliva

1.3.1 Composition

Whole saliva consists of a mixture of oral fluids, including secretions of the major and minor salivary glands, in addition to constituents of non-salivary origin derived from blood and blood derivatives, as intraoral bleeding and gingival crevicular fluid (serum exudate and inflammatory cells as leukocytes); desquamated epithelial cells as keratinocytes; other fluids as expectorated bronchial and nasal secretions; extrinsic substances as food debris; as well as microbiota as bacteria and bacterial products, viruses and fungi (Kaufman and Lamster 2000).

In human, percentage contributions of the different salivary glands during unstimulated flow are as follows: 20% from PG, 65% from SMG, 7% to 8% from SLG, and less than 10% from numerous minor glands. Stimulated high flow rates drastically change percentage contributions from each gland, with the parotid contributing more than 50% of total salivary secretions (Edgar 1990; Matsuo 2000). In addition, although most proteins in saliva are secreted by the salivary glands, there are large differences between the glands as to which proteins they synthesize (Carpenter 2013). For example, PG secretes a serous secretion that contains no mucins but is rich in amylase and proline-rich proteins (PRPs, basic and acid). Mucins and cystatins are common to the SMG and SLG as well as most minor glands. Basic PRPs appear to be exclusive to the parotid glands, whereas acidic PRPs appear in submandibular and parotid glands. However, some proteins are universal to all glands, such as IgA (the main antibody in saliva). Moreover, the composition of saliva can be also affected by physiological situations (age, sex, body weight, circadian rhythmic, etc), systemic disorders or oral diseases, stress, exercise, etc. (Edgar et al. 2012; Greabu et al. 2009). Additionally, it is important to stress that the few studies that have targeted the saliva of other mammals suggest the existence of a different proteome composition among species (de Sousa-Pereira et al. 2015; Lamy et al. 2009).

The main gland-derived salivary constituents (Kaufman and Lamster 2000) are following:

- *Water and electrolytes* ➔ Salivary fluid is composed of 99% of water (Greabu et al. 2009). However, it is composed additionally of electrolytes derived from plasma (Humphrey and Williamson 2001; Kaufman and Lamster 2000), as chloride (Cl^-), bicarbonate (HCO_3^-), sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), fluorid, thiocynate, iodine, magnesium (Mg^{+2}), phosphates, ammonia, and sulphates. Salivary fluid involves two stages. The primary fluid secreted by salivary acinar cells into the ductal lumen is a plasma-like, isotonic fluid (stage 1). Then, the striated duct cells contribute in resorbing Na^+ and Cl^- while the excretory duct cells continue the Na^+ resorption and secret K^+ and HCO_3^- (stage 2), subsequently modifying the isotonic fluid by a hypotonic one upon passage through the ducts. This is an energy rich process so that the striated duct cells have large numbers of mitochondria located in their basolateral part, leading to their description (Edgar 1990; Melvin et al. 2005). As the secretion of Na^+ and Cl^- by the acinar cells is upregulated by neural signal from the brain, but not its reabsorption by the duct cells, the stimulated saliva has a higher Na^+ and Cl^- concentration than resting saliva (Matsuo 2000).
- *Proteins*
  - *Mucin* ➔ There are two types, MG1 and MG2. MG1 is a high-molecular-weight (a molecular weight greater than 1000 kDa), highly glycosylated mucin, and forms heterotypic complexes with other salivary proteins such as amylase, PRPs, statherin, and histatins. However, the MG2 is a low-molecular-weight (with a molecular weight of 200–300 kDa), single-glycosylated peptide chain mucin (Iontcheva et al. 1997; Slomiany et al. 1996). They may be present in distinct amounts and types, and it might be the reason for the chief differences in the viscoelastic and antibacterial properties in saliva among species (de Sousa-Pereira et al. 2015).
  - *Enzymes* ➔ Saliva contains a variety of enzymes, such as lactoferrins, lysozymes, peroxidases (also known as sialoperoxidase or lactoperoxidase), glutathione-S-transferase P, sAA, esterases, among others (de Sousa-Pereira et al. 2015; Humphrey and Williamson 2001; Kaufman and Lamster 2000). All of them may be or not present in saliva from human and domestic animals, and at different concentrations. For example, the anhydrase carbonic (CA), an esterase, includes the 42-kDa CA secreted (CA VI, also named gustin), reported to be the main CA identified in human saliva, but also identified in dog, horse, cattle, sheep and rat (Asari et al. 2000; de Sousa-Pereira et al. 2015; Feldstein and Silverman 1984; Fernley et al. 1988). sAA (a-1,4-a-D-glucan 4-glucanohydrolase; EC 3.2.1.1) is one of the most important enzymes in human saliva (Nater and Rohleder 2009). Despite its concentration and activity being lower in other domestic animals, and even previously thought to be absent, it is also present in saliva from dog, horse, pig and sheep (Contreras-Aguilar et al. 2017; 2018a, b, c).

- *Immunoglobulins* ➔ Immunologic contents of saliva include secretory IgA, IgG, and IgM (Humphrey and Williamson 2001). IgA is the main antibody in saliva. It is actively carried from the connective tissues' gland across acinar and ductal cells that express the specific receptors labeled pIgR for polymeric IgA molecules, which are the prevalent form in mucosal secretions, while in serum is the monomeric form. Little or no diffusion of Igs into saliva occurs, except under conditions of inflammation or disease (Carpenter 2013).
- *Proline-rich proteins* ➔ These PRPs contribute 70% of all amino acids, with a strongly basic or acidic isoelectric point. They are highly polymorphic, with a huge variety of proteins not only between individuals but also within the same individual at different times of the day (Carpenter 2013). However, there is no evidence of them in some ruminants as sheep and goat (Amado et al. 2013; Lamy et al. 2009).
- *Cystatins, histatins and statherins* ➔ Cystatins and histatins are cysteine- rich proteins and histidine-rich proteins, respectively. Statherins are amphipathic molecules that contain both hydrophobic and hydrophilic domains, highly surface active (Carpenter 2013). Hitatins and statherins have not been found in saliva from dog, cattle, sheep, horse, rat and rabbit. However, it could be postulated that casein, observed in all the animal described above, except in human, could replace statherin and histatins as an adaptative mechanism (de Sousa-Pereira et al. 2015), since the genes encoding for this protein family belong to the same secretory gene cluster; and because its function in oral cavity is the same that cystatins, statherins, and histatins, by preventing adherence of salivary components and bacteria to enamel (Huq et al. 2005; Kawasaki and Weiss 2003).
- *Cathelicidins* ➔ Cathelicidins are antimicrobial peptides that contain a highly conserved signal sequence and cathelin domain but show substantial heterogeneity in the C-terminal dominion coding the mature active peptide (Murakami et al. 2002). They have been identified in saliva from cattle, dog, and rat (de Sousa-Pereira et al. 2015). Some studies highlight the presence of cathelicidins in human's saliva (Murakami et al. 2002), while in other they were not detected (de Sousa-Pereira et al. 2015).
- *Other proteins* ➔ In addition the main gland-derived salivary proteins, other proteins may be also found (Barranco et al. 2018; Contreras-Aguilar et al. 2019). One of these is albumin, which major route of entry into the oral cavity is via crevicular fluid (Henskens et al. 1993). Other protein that may be found in saliva is the Chromogranin A (CgA). CgA is an acidic, soluble protein, which is stored and co-released with catecholamines from granules of the adrenal medullary and sympathetic nerve chromaffin cells (Blaschko et al. 1967; Oconnor 1983); but is also produced and released from the serous acinar and ductal cells of the human SMG (Saruta et al. 2005), and also has been detected in salivary glands of rat and horse (Sato et al. 2002). In addition, there are species-specific proteins like the case of Latherin, a non-glycosylated, surface-active, detergent-like protein (Lindner et al. 2000; McDonald et al.

2009). Latherin is secreted by horses at unusually high concentrations in their sweat, to wet their hairs and facilitate the water flow for evaporative cooling, because their pelts are thick, hairy and waterproofed. Latherin has been also detected in saliva, probably as a result of an adaptative strategy to their needing to masticate and process large quantities of dry food material, and their ability to sustain high levels of exercise for long periods of time.

- *Small organic molecules* ➔ In saliva small organic molecules such as amino acids, creatinine, glucose, lipids, nitrogen, sialic acid, urea and uric acid can also be found (Eliaz Kaufman and Lamster 2000).
- *Hormones* ➔ Hormones, such as cortisol, estriol, estradiol, thyroxin and testosterone pass into saliva from plasma (Choe et al. 1983; Vining et al. 1983). All of them may reflect the levels in serum with different degree of correlation between its levels in saliva and serum. However, there are other hormones that could be produced directly by the salivary glands. For instance, there are evidences that salivary melatonin may have a local production in the salivary glands in addition to the free melatonin coming from plasma (Van Faassen et al. 2017). In addition, salivary glands during saliva production create an inactive hormone, cortisone, due to the inter-conversion of hormonally active cortisol (free serum cortisol) by the 11β-hydroxysteroid dehydrogenase type 2 (Shimojo et al. 1997). Cortisone is present at higher concentration than cortisol in saliva, and has previously been shown to have a linear relationship with serum cortisol (Debono et al. 2016). Although it has been suggested that a linear relationship exists between serum and saliva levels of insulin in humans (Marchetti et al. 1986), controversy remains as to the origin of insulin in saliva (Messenger et al. 2003) (See Chap. 8). Furthermore, other hormones related to the regulation of metabolism such as leptin, adiponectin and ghrelin, are also secreted by the salivary glands (Groschl et al. 2001, 2005; Cappai et al. 2016).

1.3.2 Function

The major functions of whole saliva are:

- *Lubrication and protection* ➔ The best lubricating components of saliva are mucins (Humphrey and Williamson 2001). Together PRPs, statherin, and histatins (Iontcheva et al. 1997), they make the saliva viscous since being of low solubility, high elasticity and strong adhesiveness. So mastication and swallowing are aided by the lubricating effects of mucins, increasing the resistance to shear at those moments (Humphrey and Williamson 2001).
- *Buffering action and clearance* ➔ In saliva, HCO_3^- is the most important buffering system. HCO_3^- is generated by the intracellular CA catalyzing the reversible reaction of water and CO_2 to form HCO_3^- and H^+ (Melvin et al. 2005). When

HCO_3^- diffuses into plaque, it acts as a buffer by neutralizing acids regulating the salivary pH: an higher flow rate will increase HCO_3^-concentrations in saliva, and salivary pH will thus be lowest (Edgar et al. 2012). In addition, HCO_3^- generates ammonia to form amines, which also serve as a buffer by neutralizing acids. After HCO_3^- activity, the major buffering ability of saliva is attributed to histatins (Mandel 1989). Urea, another buffer present in saliva, releases ammonia after being metabolized by urease activity from plaque bacteria and thus increases plaque pH (Edgar et al. 2012).

- Maintenance of tooth integrity ➔ MG1 adsorbs tightly to the tooth and thereby contributes to the enamel pellicle, which protects the tooth from acid challenges (Iontcheva et al. 1997). In addition, statherins stick tightly to the tooth surface, and together the acidic PRPs, bind to the high salivary concentrations of calcium and phosphate compared to hydroxyapatite (the main mineral component of teeth), to maintain the maturation and remineralization of enamel (Edgar et al. 2012). Therefore, they remain on the surface, bound to hydroxyapatite, to aid in controlling crystalline growth of the enamel by allowing the penetration of minerals into the enamel and by limiting mineral egress (Humphrey and Williamson 2001).
- Antibacterial activity ➔ The antibacterial activity of saliva may be specific (e.g. immunoglobulins) or non-specific (proteins, mucins, peptides, and enzymes), and it helps to control the oral microbiota (Edgar et al. 2012; Humphrey and Williamson 2001). IgA acts actively on mucosal surfaces, to neutralize viruses, serves as an antibody to bacterial antigens, and works to aggregate or clump bacteria, thus inhibiting bacterial attachment to host tissues (Humphrey and Williamson 2001; Kaufman and Lamster 2002). However, MG2 and IgA complex bind mucosal pathogens with greater affinity than either MG2 or IgA alone (Biesbrock et al. 1991). In the case of the heterotypic complexes of MG1 with sAA, PRPs, statherins, and histatins, attract the attachment of certain bacteria and providing a short-term nutrient source for bacteria (Iontcheva et al. 1997). In addition, cathelicidins act as antimicrobial peptides of the early host defenses of mammals against infection (Zanetti 2005). On the other hand, enzymes that act on the non-specific antibacterial activity are the lactoferrin, lysozymes or peroxidades (Edgar 1990; Humphrey and Williamson 2001).
- Taste and digestion ➔ The salivary hypotonicity enhances the tasting capacity of salty foods and nutrient sources (Humphrey and Williamson 2001) (see Chap. 2). Saliva has also the early, although limited, role in total digestion by beginning the breakdown of starch with sAA, the major component of parotid saliva in human that initially dissolves starch (Humphrey and Williamson 2001; Nater and Rohleder 2009). The major starch digestion results from pancreatic amylase (Butterworth et al. 2011). Other enzymes in saliva, such as lingual lipase also initiate fat digestion (Carpenter 2013; DeNigris et al. 1988). Finally, the bolus formation is another important function of saliva due to its wetting properties and its capacity to go into food to allow that the food particles to stick together once the food have been chewed and breakdown physically (Carpenter 2013).

1.4 Conclusions

The salivary gland system is a complex organ that produces saliva highly variable depending the specie and other factors related with its secretion. The secretory unit, called sialone, comprises an adenomere (secretory part) and the ductal portions that modify the product secreted by it. The regulation of salivary secretion (fluid and protein secretion) is carried out by the autonomic nervous system (sympathetic and parasympathetic) and depends on sensory, electrical and mechanical stimuli. However, nervous impulses on protein and fluid secretion from salivary glands can differ between glands in the same species and between the same gland in different species.

Whole saliva includes the secretions of the salivary glands, besides of non-salivary origin derived from blood and blood derivatives, desquamated epithelial cells, other organic fluids, extrinsic substances, as well as microbiota as bacteria and bacterial products, viruses and fungi. The main gland-derived salivary constituents are water and electrolytes, proteins (mucin, enzymes, immunoglobulins, among others), small organic molecules and hormones. The primary functions of saliva include comprise lubrication, protection, bufferin action, clearance, maintenance of tooth integrity, antimicrobial activity, taste and digestion.

References

Amado FML, Ferreira RP, Vitorino R (2013) One decade of salivary proteomics: current approaches and outstanding challenges. Clin Biochem 46(6, SI):506–517. https://doi.org/10.1016/j.clinbiochem.2012.10.024

Anderson LC, Martin DJ, Phillips DL, Killpack KJ, Bone SE, Rahimian R (2006) The influence of gender on parasympathetic vasodilatation in the submandibular gland of the rat. Exp Physiol 91(2):435–444. https://doi.org/10.1113/expphysiol.2005.032730

Asari M, Miura K, Ichihara N, Nishita T, Amasaki H (2000) Distribution of carbonic anhydrase isozyme VI in the developing bovine parotid gland. Cells Tissues Organs 167(1):18–24. https://doi.org/10.1159/000016762

Asking B, Gjorstrup P (1987) Synthesis and secretion of amylase in the rat parotid gland following autonomic nerve stimulation in vivo. Acta Physiol Scand 130(3):439–445. https://doi.org/10.1111/j.1748-1716.1987.tb08160.x

Barranco T, Tvarijonaviciute A, Tecles F, Carrillo JM, Sanchez-Resalt C, Jimenez-Reyes P et al (2018) Changes in creatine kinase, lactate dehydrogenase and aspartate aminotransferase in saliva samples after an intense exercise: a pilot study. J Sports Med Phys Fitness 58(6):910–916. https://doi.org/10.23736/S0022-4707.17.07214-0

Baum B (1993) Principles of saliva secretion. Ann N Y Acad Sci 20(694):17–23

Baum BJ, Wellner RB (1999) Receptors in salivary glands. In: Garrett JR, Ekstrom J, Anderson LC (eds) Neural mechanisms of salivary secretion, Frontiers in oral biology. Karger, Basel, pp 44–58

Beltzer EK, Fortunato CK, Guaderrama MM, Peckins MK, Garramone BM, Granger DA (2010) Salivary flow and alpha-amylase: collection technique, duration, and oral fluid type. Physiol Behav 101(2):289–296. https://doi.org/10.1016/j.physbeh.2010.05.016

Biesbrock A, Reddy M, Levine M (1991) Interaction of salivary mucin-secre- tory immunoglobulin A complex with mucosal pathogens. Infect Immun 59(10):3492–3497

Blaschko H, Comline RS, Schneider FH, Silver M, Smith AD (1967) Secretion of a chromaffin granule protein, chromogranin, from the adrenal gland after splanchnic stimulation. Nature 215:58–59

Brandtzaeg P (1998) Synthesis and secretion of human salivary immunoglobulins. In: Garrett J, Ekstrom J, Anderson LC (eds) Glandular mechanisms of salivary secretion. Karger, Basel, pp 167–199

Butterworth PJ, Warren FJ, Ellis PR (2011) Human α-amylase and starch digestion: an interesting marriage. Starch/Staerke 63(7):395–405. https://doi.org/10.1002/star.201000150

Büttler RM, Bagci E, Brand HS, den Heijer M, Blankenstein MA, Heijboer AC (2018) Testosterone, androstenedione, cortisol and cortisone levels in human unstimulated, stimulated and parotid saliva. Steroids 138:26–34. https://doi.org/10.1016/j.steroids.2018.05.013

Cappai MG, Dall'Aglio C, Sander SJ, Ratert C, Dimauro C, Pinna W, Kamphues J (2016) Different physical forms of one diet fed to growing pigs induce morphological changes in mandubular glands and local leptin (Ob) production and receptor (ObR) expression. J Anim Physiol Anim Nutr 100(6):1067–1072. https://doi.org/10.1111/jpn.12478

Carpenter GH (2013) The secretion, components, and properties of saliva. Annu Rev Food Sci Technol 4(February 2013):267–276. https://doi.org/10.1146/annurev-food-030212-182700

Carpenter GH, Garrett JR, Hartley RH, Proctor GB (1998) The influence of nerves on the secretion of immunoglobulin A into submandibular saliva in rats. J Physiol 512(2):567–573. https://doi.org/10.1111/j.1469-7793.1998.567be.x

Castle D, Castle A (1998) Intracellular transport and secretion of salivary proteins. Crit Rev Oral Biol Med 9(1):4–22. https://doi.org/10.1177/10454411980090010301

Castle J, Jamieson J, Palade G (1975) Secretion granules of the rabbit parotid gland. Isolation, subfractionation, and characterization of the membrane and content subfractions. J Cell Biol 64(1):182–210. https://doi.org/10.1083/jcb.64.1.182

Chimenos Kustner E, Ribera Uribe M, López López J (2012) Gerodontología (1ª; S. E. de Gerontología, ed.). Santiago de Compostela, España: La Ibérica

Choe JK, Khan-Dawood FS, Yusoff-Dawood M (1983) Progesterone and estradiol in the saliva and plasma during the menstrual cycle. Am J Obstet Gynecol 147(5):557–562. https://doi.org/10.1016/0002-9378(83)90016-9

Contreras-Aguilar MD, Tecles F, Martínez-Subiela S, Escribano D, Bernal L, Cerón JJ (2017) Detection and measurement of alpha- amylase in canine saliva and changes after an experimentally induced sympathetic activation. BMC Vet Res 13(266):1–6. https://doi.org/10.1186/s12917-017-1191-4

Contreras-Aguilar MD, Escribano D, Martín-cuervo M, Tecles F, Cerón JJ (2018a) Salivary alpha-amylase activity and cortisol in horses with acute abdominal disease: a pilot study. BMC Vet Res 14(156):1–7

Contreras-Aguilar MD, Escribano D, Martínez-subiela S, Martínez-Miró S, Cerón JJ, Tecles F (2018b) Changes in alpha-amylase activity , concentration and isoforms in pigs after an experimental acute stress model: an exploratory study. BMC Vet Res 14(256):1–8

Contreras-Aguilar M, Escribano D, Quiles A, López-Arjona M, Cerón J, Martínez-Subiela S et al (2018c) Evaluation of new biomarkers of stress in saliva of sheep. Animal 13(6):1–9. https://doi.org/10.1017/S1751731118002707

Contreras-Aguilar M, Escribano D, Martínez-Subiela S, Martín-Cuervo M, Lamy E, Tecles F, Cerón J (2019) Changes in saliva analytes in equine acute abdominal disease: a sialochemistry approach. BMC Vet Res 6:1–9

Davis R, Anson B, Budinger J, Kurth L (1956) Surgical anatomy of the facial nerve and parotid gland based upon a study of 350 cervicofacial halves. Surg Gynecol Obstet 102(4):384–412

de Sousa-Pereira P, Cova M, Abrantes J, Ferreira R, Trindade F, Barros A et al (2015) Cross-species comparison of mammalian saliva using an LC-MALDI based proteomic approach. Proteomics 15(9):1598–1607. https://doi.org/10.1002/pmic.201400083

Debono M, Harrison RF, Whitaker MJ, Eckland D, Arlt W, Keevil BG, Ross RJ (2016) Salivary cortisone reflects cortisol exposure under physiological conditions and after hydrocortisone. J Clin Endocrinol Metab 101(4):1469–1477. https://doi.org/10.1210/jc.2015-3694

DeNigris SJ, Hamosh M, Kasbekar DK, Lee TC, Hamosh P (1988) Lingual and gastric lipases: species differences in the origin of prepancreatic digestive lipases and in the localization of gastric lipase. Biochim Biophys Acta Lipids Lipid Metab 959(1):38–45. https://doi.org/10.1016/0005-2760(88)90147-6

Edgar WM (1990) Saliva and dental health. Clinical implications of saliva: report of a consensus meeting. Br Dent J 169(3–4):96–98. https://doi.org/10.1038/sj.bdj.4807284

Edgar M, Dawes C, O'Mullane D (2012) In: Edgar M, Dawes C, O'Mullane D (eds) Saliva and oral health, vol 56, 4th edn. https://doi.org/10.1590/S0104-42302010000100001

Ekström J (1999) Role of nonadrenergic, noncholinergic autonomic transmitters in salivary glandular activities in vivo. In: Garrett J, Ekström J, Anderson L (eds) Neural mechanisms of salivary gland secretion, Frontiers in oral biology, 11th edn. Karger, Basel, pp 94–130

Ekström J, Garrett J, Månsson B, Rowley P, G T (1989) Depletion of large dense-cored vesicles from parasympathetic nerve-terminals in rat parotid-glands after prolonged stimulation of the auriculotemporal nerve. Regul Pept 25(1):61–67. https://doi.org/10.1016/0167-0115(89)90248-6

Emilio Savastano L, Elizabeth Castro A, Rene Fitt M, Fredensborg Rath M, Eduardo Romeo H, Maris Munoz E (2010) A standardized surgical technique for rat superior cervical ganglionectomy. J Neurosci Methods 192(1):22–33. https://doi.org/10.1016/j.jneumeth.2010.07.007

Emmelin N (1987) Nerve interactions in salivary glands. J Dent Res 66(2):509–517. https://doi.org/10.1177/00220345870660022101

Emmelin N, Engstrom J (1960) Effect of sympathetic denervation on the sensitivity of the submaxillary gland to stimulating agents. J Physiol Lond 153(1):9–16. https://doi.org/10.1113/jphysiol.1960.sp006515

Feldstein JB, Silverman DN (1984) Purification and characterization of carbonic-anhidrase from the saliva of the rat. J Biol Chem 259(9):5447–5453

Fernley RT, Coghlan JP, Wright RD (1988) Purification and characterization of a high-Mr carbonic-anhydrase from sheep parotid-gland. Biochem J 249(1):201–207. https://doi.org/10.1042/bj2490201

Ferreira JN, Hoffman MP (2013) Interactions between developing nerves and salivary glands. Organogenesis 9(3):199–205

Funaki H, Yamamoto T, Koyama Y, Kondo D, Yaoita E, Kawasaki K et al (1998) Localization and expression of AQP5 in cornea, serous salivary glands, and pulmonary epithelial cells. Am J Phys Cell Phys 275(4):C1151–C1157

Greabu M, Battino M, Mohora M, Totan A, Didilescu A, Spinu T (2009) Saliva – a diagnostic window to the body, both in health and in disease. J Med Life 2(2):124–132

Gresz V, Kwon TH, Hurley PT, Varga G, Zelles T, Nielsen S et al (2001) Identification and localization of aquaporin water channels in human salivary glands. Am J Physiol Gastrointest Liver Physiol 281(1):G247–G254

Groschl M, Rauh M, Wagner R, Neuhuber W, Metzler M, Tamguney G et al (2001) Identification of leptin in human saliva. J Clin Endocrinol Metab 86(11):5234–5239. https://doi.org/10.1210/jc.86.11.5234

Groschl M, Topf HG, Bohlender J, Zenk J, Klussman S, Dotsch J et al (2005) Identification of ghrelin in human saliva: production by the salivary glands and potential role in proliferation of oral keratinocytes. Clin Chem 51(6):997–1006. https://doi.org/10.1373/clinchem.2004.040667

Henskens YMC, Vandervelden U, Veerman ECI, Amerongen AVN (1993) Protein, albumin and cystatin concentrations in saliva of healthy-subjects and of patients with gingivitis or periodontitis. J Periodontal Res 28(1):43–48. https://doi.org/10.1111/j.1600-0765.1993.tb01049.x

Humphrey SP, Williamson RT (2001) A review of saliva: normal composition, flow, and function. J Prosthet Dent 85(2):162–169

Huq NL, Cross KJ, Ung M, Reynolds EC (2005) A review of protein structure and gene organisation for proteins associated with mineralised tissue and calcium phosphate stabilisation encoded on human chromosome 4. Arch Oral Biol 50(7):599–609. https://doi.org/10.1016/j.archoralbio.2004.12.009

Iontcheva I, Oppenheim FG, Troxler RF (1997) Human salivary mucin MG1 selectively forms heterotypic complexes with amylase, proline-rich proteins, statherin, and histatins. J Dent Res 76(3):734–743. https://doi.org/10.1177/00220345970760030501

Kahle W, Frotscher M, Gerhard S (2010) Color atlas of human anatomy: nerve system and sensory organs, vol 3. Theime Medical, Stuttgart

Kaufman E, Lamster IB (2000) Analysis of saliva for periodontal sis of saliva may offer a cost-effective approach to assessment of periodontal disease in large populations. J Clin Periodontol C Munksgaard 27(27):453–465

Kaufman E, Lamster I (2002) The diagnosis application of saliva: a review. Crit Rev Oral Biol Med 13(2):197–212

Kawasaki K, Weiss KM (2003) Mineralized tissue and vertebrate evolution: the secretory calcium-binding phosphoprotein gene cluster. Proc Natl Acad Sci U S A 100(7):4060–4065. https://doi.org/10.1073/pnas.0638023100

Kim YB, Yang BH, Piao ZG, Oh SB, Kim JS, Park K (2003) Expression of Na+/HCO3- cotransporter and its role in pH regulation in mouse parotid acinar cells. Biochem BIiophys Res Commun 304(4):593–598. https://doi.org/10.1016/S0006-291X(03)00632-6

Kobashi M, Ichikawa H, Kobashi M, Funahashi M, Mitoh Y, Matsuo R (2005) The origin of sensory nerve fibers that innervate the submandibular salivary gland in the rat. Brain Res 1060(1–2):184–187. https://doi.org/10.1016/j.brainres.2005.08.012

Lamy E, da Costa G, Santos R, e Silva F, Potes J, Pereira A et al (2009) Sheep and goat saliva proteome analysis: a useful tool for ingestive behavior research? Physiol Behav 98(4):393–401. https://doi.org/10.1016/j.physbeh.2009.07.002

Lindner A, Marx S, Kissenbeck S, Mosen H (2000) Saliva collection and relationship between lactate concentration in blood and saliva of exercising horses. J Equine Vet Sci 20(1):52–54. https://doi.org/10.1016/S0737-0806(00)80322-6

Lundberg J, Anggård A, Fahrenkrug J (1981) Complementary role of vasoactive intestinal polypeptide (VIP) and acetylcholine for cat submandibular gland blood flow and secretion. II effects of cholinergic antagonists and VIP antiserum. Acta Physiol Scand 113(3):329–336. https://doi.org/10.1111/j.1748-1716.1981.tb06903.x

Luo X, Choi JY, Ko SBH, Pushkin A, Kurtz I, Ahn W et al (2001) HCO3- salvage mechanisms in the submandibular gland acinar and duct cells. J Biol Chem 276(13):9808–9816. https://doi.org/10.1074/jbc.M008548200

Mandel ID (1989) The role of saliva in maintaining oral homeostasis. J Am Dent Assoc 119(2):298–304. https://doi.org/10.14219/jada.archive.1989.0211

Marchetti P, Benzi L, Masoni A, Cecchetti P, Giannarelli R, Di Cianni G et al (1986) Salivary insulin concentrations in type 2 (non-insulin-dependent) diabetic patients and obese non-diabetic subjects: relationship to changes in plasma insulin levels after an oral glucose load. Diabetologia 29(10):695–698. https://doi.org/10.1007/BF00870278

Mathison R, Davison J, Befus A (1994) Neuroendocrine regulation of inflammation and tissue repair by submandibular gland factors. Immunol Today 15(11):527–532. https://doi.org/10.1016/0167-5699(94)90209-7

Matsuo R (2000) Role of saliva in the maintenance of taste sensitivity. Crit Rev Oral Biol Med 11(2):216–229. https://doi.org/10.1177/10454411000110020501

Matsuzaki T, Suzuki T, Koyama H, Tanaka S, Takata K (1999) Aquaporin-5 (AQP5), a water channel protein, in the rat salivary and lacrimal glands: immunolocalization and effect of secretory stimulation. Cell Tissue Res 295(3):513–521. https://doi.org/10.1007/s004410051257

McDonald RE, Fleming RI, Beeley JG, Bovell DL, Lu JR, Zhao X et al (2009) Latherin: a surfactant protein of horse sweat and saliva. PLoS One 4(5):1–12. https://doi.org/10.1371/journal.pone.0005726

Mega J, McGhee J, Kiyono H (1992) Cytokine- and Ig-producing T cells in mucosal effector tissues: analysis of IL-5- and IFN-gamma-producing T cells, T cell receptor expression, and IgA plasma cells from mouse salivary gland-associated tissues. J Immunol 148(7):2030–2039

Melvin JE, Yule D, Shuttleworth T, Begenisich T (2005) Regulation of fluid and electrolyte secretion in salivary gland acinar cells. Annu Rev Physiol 67(1):445–469. https://doi.org/10.1146/annurev.physiol.67.041703.084745

Messenger B, Clifford MN, Morgan LM (2003) Glucose-dependent insulinotropic polypeptide and insulin-like immunoreactivity in saliva following sham-fed and swallowed meals. J Endocrinol 177(3):407–412. https://doi.org/10.1677/joe.0.1770407

Mizuta K, Karita K, Izumi H (2000) Parasympathetic reflex vasodilatation in rat submandibular gland. Am J Physiol Regul Integr Comp Physiol 279(2):R677–R683

Moller K, Benz D, Perrin D, Soling HD (1996) The role of protein kinase C in carbachol-induced and of cAMP-dependent protein kinase in isoproterenol-induced secretion in primary cultured guinea pig parotid acinar cells. Biochem J 314(1):181–187

Murakami M, Ohtake T, Dorschner RA, Gallo RL (2002) Cathelicidin antimicrobial peptides are expressed in salivary glands and saliva. J Dent Res 81(12):845–850. https://doi.org/10.1177/154405910208101210

Nater UM, Rohleder N (2009) Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. Psychoneuroendocrinology 34(4):486–496. https://doi.org/10.1016/j.psyneuen.2009.01.014

Niada S, Ferreira LM, Arrigoni E, Addis A, Campagnol M, Broccaioli E, Brini AT (2013) Porcine adipose-derived stem cells from buccal fat pad and subcutaneous adipose tissue for future preclinical studies in oral surgery. Stem Cell Res Ther 4:148. https://doi.org/10.1186/scrt359

Oconnor DT (1983) Chromogranin - widespread immunoreactivity in polypeptide hormone producing tissues and in serum. Regul Pept 6(3):263–280. https://doi.org/10.1016/0167-0115(83)90145-3

Perogamvros I, Aarons L, Miller AG, Trainer PJ, Ray DW (2011) Corticosteroid-binding globulin regulates cortisol pharmacokinetics. Clin Endocrinol 74(1):30–36. https://doi.org/10.1111/j.1365-2265.2010.03897.x

Proctor G, Carpenter G (2002) Neural control of salivary secretory IgA secretion. In: Clow A, Hucklebridge F (eds) Neurobiology of the immune system. Academic, San Diego, pp 187–212

Proctor GB, Carpenter GH (2007) Regulation of salivary gland function by autonomic nerves. Auton Neurosci Basic Clin 133(1):3–18. https://doi.org/10.1016/j.autneu.2006.10.006

Roth G, Calmes R (1981) Salivary glands and saliva. In: Mosby C (ed) Oral biology. CV Mosby, St Louis, pp 196–326

Sandoval J (1998) Tratado de Anatomía Veterinaria. Tomo III: Cabeza y Sistemas viscerales, 3rd edn. Imprenta Sorles, León

Saruta J, Tsukinoki K, Sasaguri K, Ishii H, Yasuda M, Osamura YR et al (2005) Expression and localization of chromogranin A gene and protein in human submandibular gland. Cells Tissues Organs 180(4):237–244. https://doi.org/10.1159/000088939

Sato F, Kanno T, Nagasawa S, Yanaihara N, Ishida N, Hasegawa T, Iwanaga T (2002) Immunohistochemical localization of chromogranin A in the acinar cells of equine salivary glands contrasts with rodent glands. Cells Tissues Organs 172(1):29–36. https://doi.org/10.1159/000064389

Segawa A, Yamashina S (1998) The dynamics of exocytosis of preformed secretory granules from acini in rat salivary glands. In: Garrett J, Ekström J, Anderson L (eds) Glandular mechanisms of salivary secretion, Frontiers in oral biology, 10th edn. Karger, Basel, pp 80–100

Shimojo M, Ricketts ML, Petrelli MD, Moradi P, Johnson GD, Bradwell AR et al (1997) Type 2 in human mineralocorticoid target tissues: evidence for nuclear localization. Society 138(3):1305–1311

Slomiany BL, Murty VLN, Piotrowski J, Slomiany A (1996) Salivary mucins in oral mucosal defense. Gen Pharmacol 27(5):761–771. https://doi.org/10.1016/0306-3623(95)02050-0

Tamarin A (1966) Myoepithelium of rat submaxillary gland. J Ultrastruct Res 16(3–4):320–338. https://doi.org/10.1016/S0022-5320(66)80066-7

Thie NMR, Kato T, Bader G, Montplaisir JY, Lavigne GJ (2002) The significance of saliva during sleep and the relevance of oromotor movements. Sleep Med Rev 6(3):213–227. https://doi.org/10.1053/smrv.2001.0183

Van Faassen M, Bischoff R, Kema IP (2017) Relationship between plasma and salivary melatonin and cortisol investigated by LC-MS/MS. Clin Chem Lab Med 55(9):1340–1348. https://doi.org/10.1515/cclm-2016-0817

Vázquez-Autón J, Gil-Cano F, Latorre-Reviriego R, Ramírez-Zarzosa G, López-Albors O, Ayala-Florenciano M et al (2002) In: Marín D (ed) Manual de Prácticas de Anatomía Veterinaria: Sistemas Viscerales, 1st edn. Diego Marín, Murcia

Velayos J, Santana H (2007) Anatomía de la Cabeza para odontólogos (4ª). Médica panamericana, Madrid

Vining RF, Mcginley RA, Symons RG (1983) Hormones in saliva: mode if entry and consequent implication for clinical interpretation. Clin Chem 29(10):1752–1756

Young J, Cook D, Lennep E, Roberts M (1987) Secretion by the major salivary glands. In: Johnson L (ed) Physiology of the gastrointestinal tract. Raven Press, New York, pp 773–816

Zanetti M (2005) The role of cathelicidins in the innate host defenses of mammals. Curr Issues Mol Biol 7:179–196

Chapter 2
Saliva in Ingestive Behavior Research: Association with Oral Sensory Perception and Food Intake

Elsa Lamy, Ann-Marie Torregrossa, Paula Midori Castelo, and Fernando Capela e Silva

Objectives
The present chapter aims to present what is known about the involvement of saliva in the way humans and animals perceive the sensory characteristics of diet. The chapter will start by presenting the biology of flavour perception and how it influences food acceptance and choice. We will then review the newest information on the participation of saliva in flavour and taste perception and the effect of pathologies in oral sensory perception.

E. Lamy (✉)
MED - Mediterranean Institute for Agriculture, Environment and Development, IIFA - Instituto de Investigação e Formação Avançada, University of Évora, Évora, Portugal
e-mail: ecsl@uevora.pt

A.-M. Torregrossa
Department of Psychology, University at Buffalo and University at Buffalo Center for Ingestive Behavior Research, Buffalo, NY, USA
e-mail: amtorreg@buffalo.edu

P. M. Castelo
Department of Pharmaceutical Sciences, Universidade Federal de São Paulo, Diadema, Brazil
e-mail: paula.castelo@unifesp.br

F. Capela e Silva
MED - Mediterranean Institute for Agriculture, Environment and Development, IIFA - Instituto de Investigação e Formação Avançada, University of Évora, Évora, Portugal

Department of Biology, University of Évora, Évora, Portugal
e-mail: fcs@uevora.pt

A. Tvarijonaviciute et al. (eds.), *Saliva in Health and Disease*,
https://doi.org/10.1007/978-3-030-37681-9_2