Submandibular Salivary Gland Endocrine Secretions and Systemic Pathophysiological Responses

Ronald Mathison*

Department of Physiology and Pharmacology, Faculty of Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta, T2N 4N1, Canada

Abstract: Saliva is an exocrine secretion with the fluid and its components being dispensed into ducts. However, a significant component of salivary gland secretions is found in blood indicating endocrine secretion. The growth factors and hormones secreted into the blood by salivary glands manifest their actions in the face of stressful and often inflammatory stimuli, such as injury, trauma and infections, and they do not participate overtly in the regulation of the resting homeostatic steady state. This type of regulation is called allostasis, which encompasses the processes involved in maintaining systems balance in response to persistent changes, challenges, insults and injury. Viewing salivary gland endocrine secretions as having important roles in regulating responses to systemic stressors by modulating allostatic load provides a new perspective for understanding the role of salivary glands in modulation of systemic disease and pathology.

INTRODUCTION

Saliva is not one of the popular body fluids, and is only taken seriously when it disappears with ensuing difficulties in speaking and eating, necessary visits to the dentist for treatment cavities, periodontal disease, and sores in the soft tissues of the mouth. The functions of saliva, aside as a digestion aid, are thus revealed – lubrication, protection, defence and wound healing.

The good and the bad of modern medicine have made research on saliva and salivary glands both needed and respected. Endocrine disorders, stress, anxiety, depression, and nutritional deficiencies decrease saliva flow, radiotherapy for head and neck cancers are now recognized to permanently damage salivary glands, and many medications - antihypertensives, antidepressants, analgesics, tranquilizers, diuretics, antihistames and other drugs [1] leave patients with a dry mouth. Further interest in saliva has grown with its use for non-invasive diagnostic purposes for both oral and systemic diseases and the assessment of health status, treatment outcome, disease onset and progression [2]. New developments in proteomics of saliva [3] and gene transfer technologies applied to the salivary glands [4] will facilitate, respectively and cooperatively, discovery of novel therapeutic targets, development of biomarkers with diagnostic and/or prognostic value, and open up avenues to treat irreversibly damaged salivary glands and deliver protein therapeutics locally to the oral cavity, gastrointestinal tract or into the bloodstream for systemic deliver [4-6].

These new developments will also facilitate an understanding of the role of the salivary glands and their secretions in systemic health and disease. Some systemic diseases are associated with poor oral health [7], and as will be discussed subsequently, the proteins and peptides synthesized in the salivary glands that are secreted into the blood have subtle, but significant impact on systemic response to a variety of stresses and pathologies. The functions of these salivary endocrine factors and their involvement in the regulation of systemic response to stressors are interpreted within the concept of allostasis and allostatic load.

SALIVA AND THE SALIVARY GLANDS

Saliva, an exocrine secretion of the salivary glands, consists of water, electrolytes, enzymes, immunoglobulins, mucosal glycoproteins and numerous antimicrobial proteins, enzymes, growth factors and regulatory peptides. These components aid in the sensation of taste, digestion of starch and lipids and are responsible in the maintenance of oral health by protecting tissues with lubrication and buffering properties, contributing to the physical-chemical integrity of tooth enamel, and preventing adhesion of and colonization by microorganisms.

Saliva is produced by major (parotid, submandibular, and sublingual) and minor (located throughout the oral cavity) salivary glands. The salivary glands are composed primarily of ductal and acinar cells, with the latter consisting of serous cells that produce a water saliva (e.g. parotid), or mucous cells (e.g. sublingual and minor salivary glands) which yield a secretion rich in immunoglobulins and mucus. The submandibular glands have both serous and mucous acinar cells. The ductal cells line the salivary ducts leading from the acinar cells to the oral cavity and modify salivary composition by absorbing sodium chloride, and by adding proteins, growth factors and potassium. Salivary secretion is under autonomic nervous system control [8] with parasympathetic nerves activating the muscarinic receptors responsible for fluid secretion and the sympathetic nerves through stimulation of adrenergic receptors regulating protein secretion.

*Address correspondence to this author at the Department of Physiology and Pharmacology, Faculty of Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta, T2N 4N1, Canada;
E-mails: rmathiso@ucalgary.ca, salpep@shaw.ca

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Relationship to Systemic Health

The relationship between salivary glands and systemic health is complex, involving several bidirectional interacting factors. Currently, a great deal of evidence suggests that poor oral health can contribute to systemic disease, and the opposite is equally true. Chronic inflammatory autoimmune disorder of the salivary glands yields compromised secretory function, which impacts on peripheral systems. Systemic autoimmune diseases also can modify salivary gland function and oral health. The relationships are complex, and only recently have attempts been made to unravel and reveal causal relationships.

Periodontal Disease and Systemic Health

Chronic inflammatory periodontal diseases are among the most prevalent chronic infections in humans, and many investigators have established a significant, albeit modest, positive association between periodontal disease and cardiovascular disease, which includes atherosclerosis, myocardial infarction and stroke. In addition, epidemiological associations have been made between periodontal diseases and chronic diseases such as diabetes, respiratory diseases and osteoporosis [9]. Nonetheless, the association between periodontal disease and systemic disease is not always accepted, and the call has been made for prospective studies to evaluate this relationship with a rigorous assessment of both clinical endpoints and surrogate markers of risk [10]. Despite the uncertainty it is recognized that ``oral infection may represent a significant risk-factor for systemic diseases, and hence the control of oral disease is essential in the prevention and management of these systemic conditions`` [10]. A clear definition of biological mechanism is still required and several hypotheses have been presented: 1) contribution to systemic inflammation with increased circulating cytokines and mediators; 2) cross-reactivity or molecular mimicry between bacterial antigens and self-antigens; and 3) direct infection of the blood vessel walls by periodontal organisms that escape into the circulation [9, 11]. Another mechanism could involve modification by oral infections of the secretion of salivary factors (Table **1**) that impact on systemic health. This mechanism has received little attention and is evaluated herein.

Systemic Diseases Associated with Hyposalivation and/or Xerostomia

Broadly speaking salivary dysfunction manifests as either hypersalivation (sialorrhea) or hyposalivation. Xerostomia, which refers to a subjective experience of mouth dryness, is often used interchangeably, but incorrectly, with hyposalivation. Patients with hyposalivation are not necessarily xerostomic, and may have normal salivary flow rates. Nonetheless, salivary gland hypofunction is a common and primary symptom of xerostomia. Hypersalivation may be caused by drugs having cholinergic effects, and is often related to neurological disorders such as Parkinson's disease, amyotrophic lateral sclerosis (ALS) and developmental disabilities (e.g., cerebral palsy; Down syndrome, fragile X, autism).

Hyposalivation can be induced by medications, chemo- and radiotherapy, and graft-versus-host disease (GVHD) that cause significant oral pathology with mucosal infections dental caries, and difficulties in tasting, eating, swallowing, and speaking. These pathologies also manifest in patients with a variety of systemic diseases including – SS (Sjögren's syndrome), rheumatoid arthritis, juvenile idiopathic (rheumatoid) arthritis, systemic lupus erythematosus (an inflammatory connective tissue disease), systemic sclerosis (scleloderma), primary bilary cirrhosis (an autoimmune disease of the liver), sarcidosis (a multisystem granulomatous disorder), infections with human immunodeficiency virus (HIV), herpes virus, hepatitis C, ectodermal dysplasia, chronic pancreatitis and depression [7].

Table 1. Some Salivary Gland Growth Factors, Enzymes and Peptides that Impact on Systemic Health

Factor, Enzyme or Peptide	Abbreviation
Basic fibroblast growth factor	bFGF
Brain-derived neurotrophic factor	BNDF
Epidermal growth factor	EGF
Hepatocyte growth factor	HGF
Insulin-like growth factors I and II	IGF-I & IGFII
Kallikrein	
Nerve growth factor	NGF
Platelet derived growth factor	PDGF
Submandibular gland peptide-T (TDIFEGG)	SGPT
Sialorphin (QHNPR)	
Submandibular rat-1	SMR1
Renin	
Transforming growth factor alpha	TGFα
Transforming growth factor beta	TGFβ
Vascular endothelial growth factor	VEGF

The mechanisms responsible for hyposalivation include [7]: 1) neurotransmitter receptor dysfunctions, as seen with the presence of IgG antibodies M_3 and M_1 mAChRs in patients with SS [12, 13], and lack of stimulation of nitric oxide synthase activation in the submandibular glands by vasoactive intestinal peptide (VIP) in non-obese diabetic (NOD) mice [14]; 2) alterations of fluid composition and electrolytes, as occurs in SS patients with reduced concentration of nitrite, a cytocidal and cytostatic agent in human saliva that kills several oral pathogens [15]; 3) DNA damage (radiation therapy); 4) immune dysregulation associated with salivary gland parenchymal destruction, as with GVHD [16] and SS [17]; and 5) a combinations of these mechanisms.

SJÖGREN'S SYNDROME - A SALIVARY GLAND DISEASE WITH SYSTEMIC MANIFESTATIONS

Sjögren's syndrome is a slowly progressive chronic inflammatory autoimmune disorder associated with autoimmune destruction of the exocrine glands that occurs almost exclusively in women (>90%). SS may be either primary, accounting for approximately 50% of the cases, or secondary occurring in association with other connective tissue diseases, most commonly rheumatoid arthritis or systemic lupus

erythematosus. This disease is characterized by failure of exocrine secretion from salivary and lacrimal glands. The manifesting features of SS are severe dryness of the mouth (xerostomia) and eyes (keratoconjunctivitis sicca) with many patients developing extraglandular manifestations features involving the kidney, liver, lungs, thyroid and the gastrointestinal tract [18]. Myalgias (muscle pain) and arthralgias (joint pain), and variable alterations in the sensory and autonomic nervous system have been reported [19-21].

The respiratory manifestations of SS include interstitial pneumonitis, "small airways disease" and pleuritis, with one-third of the patients exhibiting chronic, dry non-productive cough and dyspnoea [22]. Close to 60% of the patients with primary SS have bronchial hyperresponsiveness (BHR) to methacholine [22-24], but atopy (allergic reaction involving immunoglobulin E) is not overly expressed. Although the mechanism for increased BHR has not been extensively investigated some precipitating causes could be dryness of the respiratory mucosa, epithelial damage or increased inflammatory cells in the airway mucosa, as there appears to be an involvement of neutrophils, mast cells and T-lymphocytes [22, 25]. This cellular profile is different from that seen with atopic asthma, because corticosteroids, which effectively alleviate BHR and respiratory symptoms in asthmatic patients, are in ineffective in SS [26]. In addition, SS patients do not exhibit BHR to adenosine 5'-monophosphate (AMP) as do atopic subjects [27].

Because there are many exocrine glands in the gastrointestinal tract SS can involve any part of the gut, including the salivary glands, mouth, oesophagus, stomach, pancreas, hepatobiliary tree, and large and small bowel [28]. Mucosal atrophy can also be seen throughout the entire length of the oesophagus [29]. Chronic atrophic gastritis [30], duodenal ulcers [31] and mucosa-associated lymphoid tissue lymphomas within the gastrointestinal tract [28] are problematic in SS. Nearly 25% and 50% of SS patients have, respectively, autoimmune pancreatitis [32] and abnormal liver function [33].

Potential mechanisms for SS-associated dysfunctions include [19]: 1) T-cell infiltration and destruction of ganglions and nerves, 2) cytokine-induced inhibition of neuropeptide secretion from nerve endings, 3) immune complex-mediated inflammation, and 4) pathogenic autoantibodies, possibly an autoimmune epithelitis [18]. There is currently no cure for SS, and treatment is mainly palliative with intense oral hygiene, prevention and treatment of oral infections, use of saliva substitutes, and local and systematic stimulation of salivary secretion. Cholinergic agents are the cornerstone of current pharmacotherapy, with corticosteroids, cyclophoshamide, and nucleoside analogues being used only in patients with severe extraglandular manifestations of the disease [34].

A significant problem with studying SS and linking cause to symptoms is the silent asymptomatic phase of the disease initiation and development. Several recent advances may help resolve this issue. Mouse models of human SS, such as the NOD and the MRL/*lpr mice*, first used, respectively as models for type I diabetes and systemic lupus erythematosus [35, 36], develop SS- like lesions in their salivary and lacrimal glands. Also, proteomic analysis may offer scope for treatment modalities. With SS patients the expression of two trophic factors, EGF and TGFα, are diminished in labial salivary glands [37], and tear fluid EGF concentration decreases as levels of inflammatory cytokines in the conjunctival epithelium increase [38]. Saliva from SS patients reveals a set of differentially expressed proteins relative to controls that relate to active and chronic inflammation (e.g. ß2-microglobulin, the small subunit of the MHC class I molecule; cyclophilin, involved in immune-mediated endothelial activation and dysfunction; and Calgranulin B, a member of S100 family of calcium binding proteins present in both acute and chronic inflammation), while some others are involved in oxidative stress injury (e.g. glutathione S-transferase, involved in limiting oxidative injury; lipocalin, the lipocalin A precursor, a physiological scavenger of potentially harmful molecules derived from lipid peroxidation; and epidermal fatty acid binding protein (E-FABP) [39, 40]).

To date no studies have evaluated endocrine secretion of hormones or growth factors from salivary glands in patients with SS or sialadenitis. The following discussion of endocrine secretions from salivary glands of rodents in modulating systemic diseases and pathologies suggests that this aspect may need consideration for a better understanding of the systemic (non-autoimmune) complications of SS.

Endocrine Secretion from Salivary Glands

The salivary glands are generally considered as exocrine glands that dispense their protein and fluid externally into a lumen or a duct. However, investigations dating from 60 years ago suggested an unorthodox view that salivary and other exocrine glands, such as the pancreas, are capable of endocrine secretion, dispensing their secretions internally, i.e. directly into the blood stream. It has been suggested that these glands be called “duacrine” glands [41]. With both types of secretion the secreted proteins originate in the *trans*-Golgi network and are, for the most part, stored at high concentrations in dense core secretory granules [42], and leave the cells *via* the exocytotic (regulated) pathway. Granule movement for endocrine secretion is to the blood-facing basolateral membrane surface, whereas with exocrine secretion the granules move towards the duct-facing apical membrane.

Salivary glands, of both rodents and humans, synthesize and release several biologically active peptides and hormones, including EGF, nerve growth factor (NGF), transforming growth factor-alpha and -beta (TGF-α and TGF-β), hepatocyte growth factor (HGF), insulin, insulin-like growth factors I and II (IGF-I, IGFII), and basic fibroblast growth factor (bFGF) [43] (Table **1**). The physiological roles of these factors in the oral cavity are not completely understood, although they generally seem to be involved in salivary gland morphogenesis, wound healing and tissue regeneration [44, 45], and are important factors in the etiology of oral and glandular inflammation and malignancies [46-48] (Fig. **1**). Secretion from rodent submandibular glands into the blood has been demonstrated for glucagon [49], EGF [50], NGF [51], renin [52], kallikrein [53] and sialorphin [54-56]. Endocrine secretion from the parotid has been shown for parotin, a protein complex originally extracted from bovine parotid glands [57], and amylase [58]. These biologically active polypeptides modify a variety of functions including growth and differentiation, enzymatic control, homeostatic regulation, and adaptation to stress [59-61].

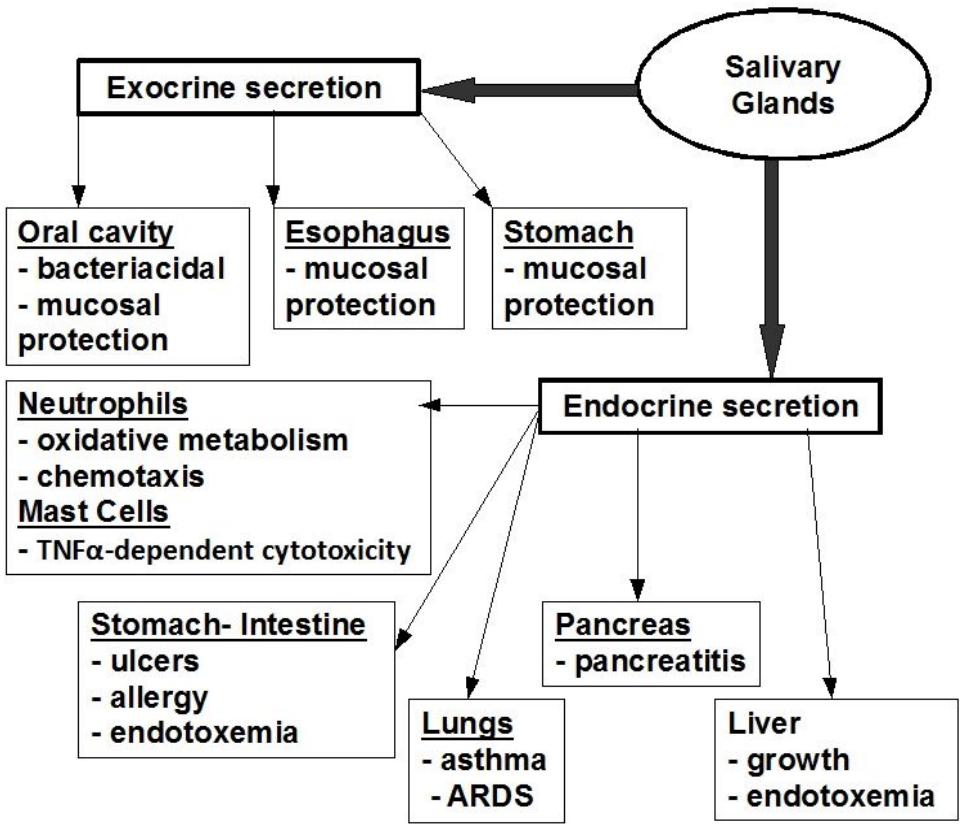

Fig. (1). Exocrine and Endocrine Secretion from Salivary Glands. Several functions of organs, tissues and cells regulated by exocrine and endocrine secretion of peptides and growth factors from salivary glands. A decrease or loss of salivary gland endocrine secretion results in inappropriate or pathological responses of these tissues to various stressors.

Recent Identified Salivary Gland Peptide Hormones

A recent addition to the salivary gland family of regulatory peptides are the peptides derived from the 146 amino acid prohorome SMR1 (submandibular rat-1), which is a product of the variable coding sequence-a1 (submandibular rat-1) gene [62]. SMR1 is predominantly expressed in the acinar cells of the submandibular gland of male rats and in the prostate [63, 64], and is sex-linked with differential expression occurring essentially through transcriptional and/or post-transcriptional regulation exerted by androgens [61].

Proteolytic processing of the SMR1 prohormone [54] yields small peptide hormones, two of which have received the most attention (Fig. **2**). The pentapeptide (QHNPR, sialorphin) is secreted into the bloodstream of male rats in response to acute stress and adrenergic stimulation, and is selectively taken up by peripheral targets [54, 56, 61]. Sialorphin is functionally related to the peptide QRFSR, an inhibitor of two enkephalin-catabolizing ectoenzymes - human neutral ecto-endopeptidase, hNEP (EC 3.4.24.11), and human ecto-aminopeptidase, hAP-N (EC 3.4.11.2) [65]. The relationship between actions on ectoenzymes, and the ability of sialorphin to modulate the adaptive balance between excitatory and inhibitory mechanisms serving appropriate male rat sexual response [66] has not been established.

The C-terminal of SMR1 prohormone also contains a biologically active heptapeptide, submandibular gland peptide-T (SGPT; TDIFEGG; aa 138-144) [67-70]. From sequential alanine substitutions in SGPT, the tripeptide FEG and the dipeptide FE were identified as minimal sequences that reduced the severity of allergic reactions [68, 71], although it is not known if these peptide fragments of SMR1 prohormone are endogenous to the salivary glands. The D-isomer of FEG (D-phe-D-glu-Gly; feG) mimics that actions of feG [72]. The SMR1-derived peptide hormones will be discussed in more detail below.

Submandibular Glands Peptides and Systemic Pathology

Endotoxemia

Submandibular glands are required for processes involved in liver cell proliferation. If these glands are removed liver regeneration after partial hepatectomy is compromised [73, 74], and the livers respond to a carcinogenic agent with fewer adenomatous nodules and carcinomas than control mice [75]. The liver of sialoadenectomized mice appears normal and have normal liver glycogen and plasma glucose concentration after immobilization, but after an aggressive encounter the hepatocytes are reduced in number but have an increased volume [76]. From these and other observations the concept of a submandibular gland-liver axis in rodents has been proposed [76]. Support for this concept was obtained when Buira and coworkers showed that sialoadenectomy induced transient apoptotic cell death, an increase in DNA synthesis with an increase in cell volume, but cell division was unaltered. These changes did not affect liver cell responses to stress (immobilization, aggressive encounter and fasting), but sialoadenectomy of mice receiving a non-lethal dose of bacterial lipopolysaccharide (LPS) combined

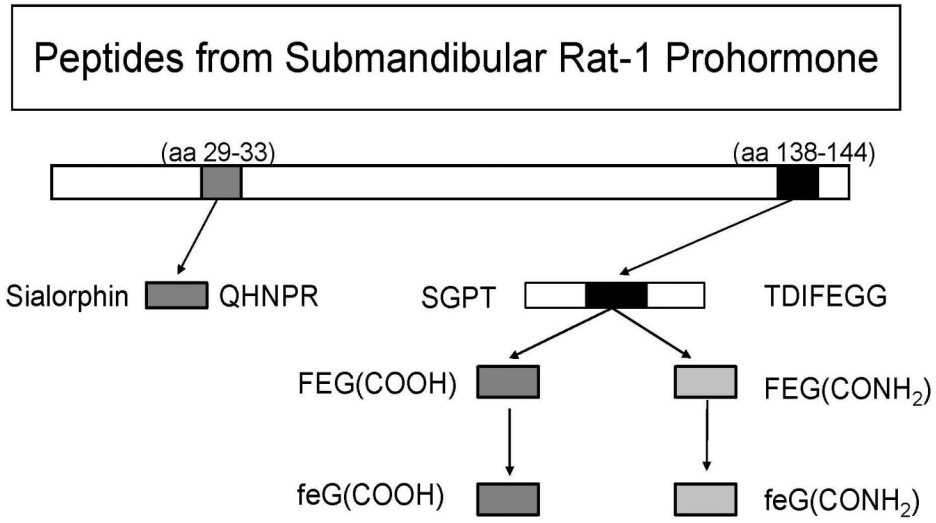

Fig. (2). The SMR1 (Submandibular Rat-1 Prohormone) precursor protein is a prohormone that contains sialorphin near the N-terminal, and SGPT (submandibular gland peptide T) near the C-terminal. Sialorphin is a neutral endopeptidase inhibitor and also relaxes corporal smooth muscle. The peptide FEG(COOH) possesses biological activity in allergic and neutrophil-mediated inflammation, whereas its carboxyamide derivative (FEG(CONH2) is effective against endotoxic reactions. The D-amino acid derivatives of feG and feG(NH_2) are biologically active, although they are not present endogenously in the salivary glands.

with D-galactosamine, resulted in increased plasma alanine aminotransferase and aspartate aminotransferase, and liver myeloperoxidase (MPO) activities [76]. These effects of sialoadenectomy on the endotoxic reaction are a consequence of an inadequate cytokine production by the liver and a reduced corticosteroid release from adrenal glands [77].

A disturbance of the submandibular gland-liver axis induces an adaptive response that preserves the metabolic function of the liver but renders it more sensitive to bacterial endotoxins [77]. Although EGF is apparently the salivary gland factor contributing to liver regeneration [78], this growth factor does not contribute to endotoxin-induced hepatotoxicity [77].

Removal of the submandibular glands also increases the hypotensive responses to endotoxin [67], and a putative candidate for the protective factor released from these glands is the carboxyl-terminus amidated derivative of the C-terminal peptides of SMR1 protein (i.e. TDIFEGGGK; Fig. **2**). The peptide mimic feG($CONH_2$), but not feG-COOH, reduces the severity of endotoxic hypotension, endotoxin-induced perturbation of intestinal motility [79], and reduces adhesion of leukocytes to extravascular tissue [80]. However, the effect of feG($CONH_2$) on endotoxin hepatic damage remains to be evaluated. One would expect that TDIFEGGGK is acted upon by a carboxypeptidase B-like enzyme removing a basic residue (Lys (K)), and then further action by peptidylglycine alpha-amidating mono-oxygenase (PAM), which has two catalytic domains working sequentially on glycine-extended peptides [81] would generate an alpha-amidated peptide (e.g. TDIFEGG-($CONH_2$). feG($CONH_2$) acts as a mimic of alpha-amidated SGPT, which when released by endocrine secretion into the blood stream acts as a regulator of endotoxic events. PAM has been identified in salivary secretions in rats [82], and this type of enzymatic processing is common and used for full activation of more than 45 other bioactive peptides including substance P, gastrin, oxytocin, and adrenocorticotropic hormone [81, 83].

Allergic Reactions

Denervation of the salivary glands by removal of the superior cervical ganglia was found to reduce the severity of pulmonary inflammation initiated by systemic anaphylaxis [84]. Although sialoadenectomy did not modify the severity of the pulmonary inflammation concurrent sialoadenectomy and decentralization abolished the protective effect of decentralization [85]. From these observations we postulated that cervical sympathetic nerves tonically inhibit release of anti-inflammatory factors from submandibular glands, and the heptapeptide SGPT was subsequently isolated from rat salivary glands [67]. The tripeptide FEG, found in the C-terminal sequence of SGPT (TDI**FEG**G) and its metabolically stable D-isomeric analogue, feG, exerts anti-allergic activities in mice, rats, sheep, cats and dogs [72, 86, 87]. feG also acts on isolated human leukocytes [88] indicating that receptor sites for this peptide are present in all species studied to date. feG, as a potent inhibitor of IgE-mediated allergic reactions, reduces the amplitude of intestinal [72, 89], and bronchiolar smooth muscle contractions [90], bronchoconstriction, late-phase hypersensitivity reactions [87, 90] and pulmonary inflammation [86, 90].

The anti-inflammatory actions of feG involve:

1. modulation of leukocyte adhesion by actions on αMβ2 integrin, with a possible interaction with the low affinity FcγRIII receptor (CD16) [91];
2. prevention of inflammation-induced up-regulation of σ4β1 integrin (VLA4; CD49d) on circulating neutrophils [92], and
3. reduced generation of reactive oxygen species by neutrophils by a mechanism involving the regulation of protein kinase C (PKC) [92]. This latter action of feG also accounts for its ability to reduce the severity of neutrophil-mediated tissue damage following spinal cord injury [93].

Gastrointestinal Ulcers

A variety of factors play a role in the development of stomach or duodenal ulcers. These include: *Helicobacter pylori*, smoking, caffeine, alcohol, stress, nonsteroidal anti-inflammatory drugs (NSAIDs). The link between gastric ulcers and salivary glands occurred with the discovery of EGF [94, 95], a 53 amino acid protein with three intramolecular disulfide bonds, that is involved cell growth, proliferation and differentiation, and plays a role in oncogenesis and wound healing. Early on it was discovered that EGF, then also known as uragastrone, inhibited gastric acid secretion [96] and promoted the growth of the oxyntic (acid producing) mucosa of the stomach [97]. Since EGF was isolated originally from the submandibular glands of mice [95], and given its effects on the gastric mucosa it was noticed that removal of these glands exacerbated ethanol-induced gastric ulcers [98]. The protection afforded by the submandibular glands against ulcer formation is seen with a large number of ulcerogenic agents and situations, such as acetic acid, dexamethasone, cysteamine, indomethacin and water-immersion restraint stress. In rats with their submandibular glands removed the increased susceptibility to ulcerogenic agents is of long duration, lasting up to 200 days [99]. These glands help maintain mucosal integrity by promoting blood flow and growth of the mucosal coat by stimulating synthesis of mucin, and enzymes favourable to ulcer healing (e.g. nitric oxide synthase and cyclo-oxygenases) [97, 100, 101]. The protective effects of the submandibular gland on the stomach extend beyond healing of ulcers as these glands are required for the induction and maintenance of optimal immunity against *Helicobactor pylori*, an ulcer causing bacteria [102]. On the negative side, the presence of the submandibular glands promotes the growth of xenografted tumours [103].

A large number of cytokines and growth factors (EGF, PDGF, HGF, TGFβ, VEGF, angiopoietins) are involved in gastrointestinal maintenance and ulcer healing, and the relative importance of salivary gland EGF for this healing in humans has not been ascertained. Initial claims that salivary gland factors promote mucosal healing in gastroesophageal reflux disease have not been confirmed [104]. The multifactorial nature of ulceration, and the difficulties in selective manipulation of salivary function in humans impose constraints in discerning whether a deficiency in one or more salivary growth factors might be a contributing factor to ulcer prevention in the human gastrointestinal tract. Nonetheless, some insight into the role of salivary glands in gastrointestinal ulceration may be gained by considering patients under intensive medical care such as those undergoing haemodialysis, which has significant acute effects on both salivary secretion rate and protein concentrations in saliva [105]. Haemodialysis patients are prone to developing peptic ulcers and have significant periodontal disease. Two studies have shown that these patients have decreased amounts of salivary EGF [106] and HGF [107]. The decreased bioactivity, but not total amounts measured by HPLC, of salivary EGF [106] illustrates that a measurement of factor bioactivity rather than just amounts by immunological or chemical assays may eliminate a confounding variable in these types of studies.

Pancreatitis

The salivary glands and pancreas have many histological and functional similarities. Salivary gland function is frequently impaired with chronic pancreatitis of various etiologies, including autoimmune and idiopathic chronic pancreatitis [108, 109]. An immune response directed against the pancreatic ductal system also adversely affects the salivary ducts [108].

Acute pancreatitis is an inflammatory disease of the pancreas. Common symptoms are acute abdominal pain with diagnositic confirmation made with increased concentrations of serum amylase and lipase. In most patients (~80%) pancreatic injury is mild without complications and treatment is supportive. However, local and systemic complications associated with severe disease are a medical emergency [110], as systemic inflammatory response syndrome (SIRS) often develop to manifest in life-threatening acute respiratory distress syndrome (ARDS) [111].

In experimental studies, salivary gland removal did not affect significantly the histological signs of pancreatitis induced by caerulein (10 μg/kg/h for 5 h), but resulted in an enhanced reduction in pancreatic blood flow and DNA synthesis and an increase in plasma interleukin-1β [112]. EGF is generally selected as the salivary gland factor contributing to adverse effects of salivary gland removal, and indeed exogenous EGF enhanced pancreatic recovery after caerulein-induced pancreatitis, and prevented the pathological changes associated with salivary gland removal [112]. Salivary gland removal does not affect pancreatic blood flow in normal healthy animals [113], which is consistent with other observations that salivary glands do not participate in maintaining normal systemic homeostasis, but rather participate in reducing provoked responses. In keeping with these conclusions are the observations that prophylactic treatment of rats with the salivary gland-derived peptide mimetic, feG, significantly reduced the increases in plasma amylase (45%), pancreatic histology (30%), myeloperoxidase - a measure of neutrophil infiltration (80%) and ICAM-1 mRNA expression (50%) caused by caerulein-induced pancreatitis, but did not modify these parameters in normal animals [114]. The reduction of neutrophil infiltration associated with pancreatic injury reflects the inhibitory effects of feG on neutrophil migration and activation seen in IgE-mediated hypersensitivity reactions [80, 88, 91, 92] and spinal cord injury [93].

Thus, several salivary gland factors (EGF and FEG-analogues) appear to contribute to pancreatic tissue protection in response to injury by maintaining blood flow, preventing necrotic damage and reducing activation of inflammatory cascades.

Stressors

Numerous studies have established that the submandibular glands participate in stress responses such as aggressive behaviour, exposure to ether fumes, water immersion and restraint stress. These stressors cause the endocrine release from submandibular glands of a variety of factors, including renin [115], EGF [116], NGF [117], BNDF (brain-derived

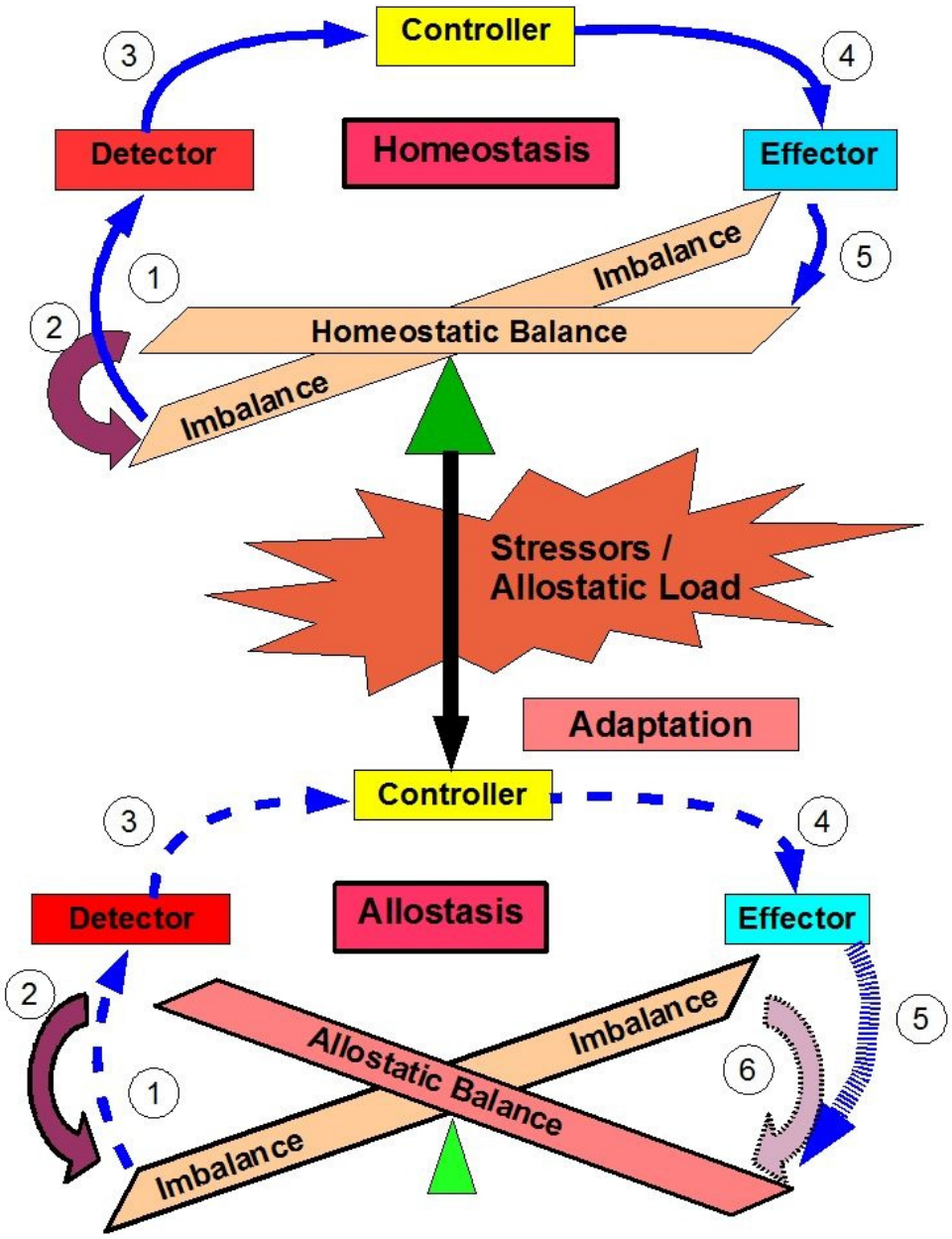

Fig. (3). Allostasis and Allostatic Load. The top diagram illustrates a homeostatic process whereby a stimulus causing an imbalance (1) produces a change (2) in a variable that is detected by receptor. This change or input signal (3) is sent to a control center along an afferent pathway, and after integration an output signal (4) is sent to an effector along an efferent pathway. The effector feeds back to modulate stimulus intensity (5) and returns variable to normal and homeostasis is re-established. The lower diagram shows that with stress or allostatic load (the cumulative response to ongoing demands for physiological change) an adaptation occurs such that a modified state develops (allostasis) with the establishment of an allostatic balance. The adaptive changes result in modified signalling (1), detection (2), transmission ((3 & 4), controller or effector (5) systems such that imbalance is not corrected properly (6), since the allostatic state is unable to respond or gives an inappropriate response to an additional or new stressors.

neurotrophic factor) [118], and sialorphin [61]. Rats with their submandibular glands removed show an increased severity of the gastric ulceration induced by water immersion and restraint stress [119].

The biological role of these submandibular factors in the response to stress is poorly understood, but several salient suggestions have been made. NGF possesses anti-inflammatory actions [120], and probably participates in the repair and remodelling of damaged tissue following acute and/or chronic stressful events by promoting repair of damaged nerve cells [121], and improving the healing of cutaneous ulcers [122, 123]. Similarly, stress-induced release of EGF from salivary glands may accelerate wound healing, and provide protection of the gastrointestinal tract from ulcerogenesis [124].

This discussion on stressors and the preceding sections on aggravated pathology in sialoadenectomized animals to inflammatory stimuli (endotoxin and allergens) and ulcerogenic stimuli emphasize that salivary glands and their endocrine secretions ``might participate in integrative re-

establishments of dynamic homeostatic responses to severe (stressful) physiological situations following injury, trauma or infection, rather than contribute to the regulation of resting (unstimulated) homeostatic steady state`` [61]. This type of homeostatic regulation is called allostasis. In the next section, the concept of allostasis is briefly summarized and the proposal is put forward that the salivary glands, in particular the submandibular glands, regulate responses to systemic stressors consequent to their modulation of allostatic load (Figs. **3**, **4**).

ALLOSTASIS AND ALLOSTATIC LOAD

The term allostasis, coined in 1988 by Sterling and Eyer [125], refers to the active processes by which the body responds to challenges and changes made to achieve and maintain homeostasis. Homeostasis, a concept introduced by WB Cannon in 1932, focuses on the systems essential for life and describes the property of a living organism to regulate its internal environment so as to maintain a steady state (e.g. blood pressure, blood glucose). Allostasis is more dynamic and focuses on the processes required to maintain systems in balance in the face of environmental, life stage and unexpected, sometimes persistent, changes or challenges [126]. The adaptive physiological and behavioural modifications driven by allostasis will change the set-points and boundaries of homeostatic processes.

Normally an allostatic response, initiated by a stressor, is sustained for an appropriate interval, and then turned off when adaptation is achieved. The concept of allostatic load was proposed to account for the "the wear and tear that the body experiences due to repeated cycles of allostasis as well as the inefficient turning-on or shutting off of these responses" [127] (Fig. **3**). Thus, allostatic load is the cumulative response to demands for physiological change, and four types of conditions leading to allostatic load have been delineated [128]. These four types of allostatic load are:

1) Repeated hits from stressors. High allergen burden increased the odds of having asthma symptoms [129], and chronic damage in the pancreas may result from repeated attacks of acute tissue inflammation caused by alcohol abuse [130]. It was noted more than a decade ago that repeated exposures of rats to water immersion and restraint stress over 6 days leads to the adaptation of the gastric mucosa to stress ulcerogenesis [131]. Removal of the submandibular glands, which significantly lowered stomach luminal EGF, delayed and reduced this adaptation, an effect that was reversed by treatment with exogenous EGF [131].

2) Lack of adaptation resulting in prolonged exposure and inadequate response to a stressor. This lack of adaptation could be a consequence of a genetic defect, for example the defective neurotransmitter-mediated signalling in the salivary glands of NOD mice contributes to the development of sialadenitis and eventually SS [132]. Removal of the submandibular glands results in several pathologies possibly related to an inadequate response to a stressor. Sialoadenectomy renders the gastrointestinal tract more sensitive to ulcer-inducing agents [98, 99, 101, 133-136], alters reproductive function, both in male [137, 138] and in female [139] mice, renders rats more sensitive to a endotoxic-induced hypotension [140], liver damage [77] and IgE-mediated hypersensitivity reactions [85] and enhances the response of adipocytes [141], hepatocytes [142], and cardiac myocytes [143] to catecholamines. Another example of lack of adaptation is differential responses of some patients to NSAIDs. With most, but not all, patients the gastrointestinal damage resolves by a process of adaptation, which may be associated with an increase EGF in saliva [144] and gastric juices [145].

3) Prolonged response due to delayed shut down or an inability to shut off allostatic responses after a stress is terminated. Examples are the absence of a recovery in blood pressure in some people after an acute stress and hypertension accelerated atherosclerosis [128]. The induction of a self-sustaining loop of immune-nonimmune interactions has been proposed as a mechanism that contribute to the persistence of inflammatory bowel disease [146].

4) Inadequate response that leads to compensatory hyperactivity of other mediators. A classical example is inadequate secretion of glucocorticoid, resulting in increased levels of cytokines that are normally counter-regulated by glucocorticoids [127]. A salivary gland counterpart to an inadequate response may exist for the development and maintenance of SS, which is considered to be a T-cell-mediated autoimmune disease. Patients with this disorder harbour unique and highly selected T- and B-cell populations. An immune dysregulation or inappropriate development of tolerance could lead to the presence of autoreactive and hyporeactive B-cells contributing to the generation of autoantibodies that eventually lead to a autoimmune chronic inflammatory pathology [147].

A putative role for salivary glands in modulating responses to allostatic load involve adaptation to repeated hits from stressors (type 1), and inadequate response to a stressor (type 2), and an inadequate response that leads to compensatory hyperactivity of other mediators (type 4) may be a characteristic of human SS.

Salivary Glands and Adaptation to Stress

Primary mediators of allostasis are hormones of the hypothalamo–pituitary–adrenal (HPA) axis, neurotransmitters of the autonomic nervous system, cytokines and regulators of oxidative stress [148]. Salivary cortisol, as an endocrine marker of HPA-axis activation, is a non-invasive measure allowing for repeated and simple stress-free sampling that is used for investigating adrenal function in a variety of disciplines and has been applied in studies examining psychosocial stress and allostatic load [149].

In addition to salivary gland growth factors and peptide hormones that modulate systemic responses to allostatic load, interactions with other regulatory systems should also be considered (Fig. **4**). For example, gastric adaptation to stress ulceration is not altered by vagotomy or adrenalectomy, but is dependent upon an intact sensory nervous system [124]. The network of endocrine factors involved in maintenance of a healthy mucosa and in ulcer formation is

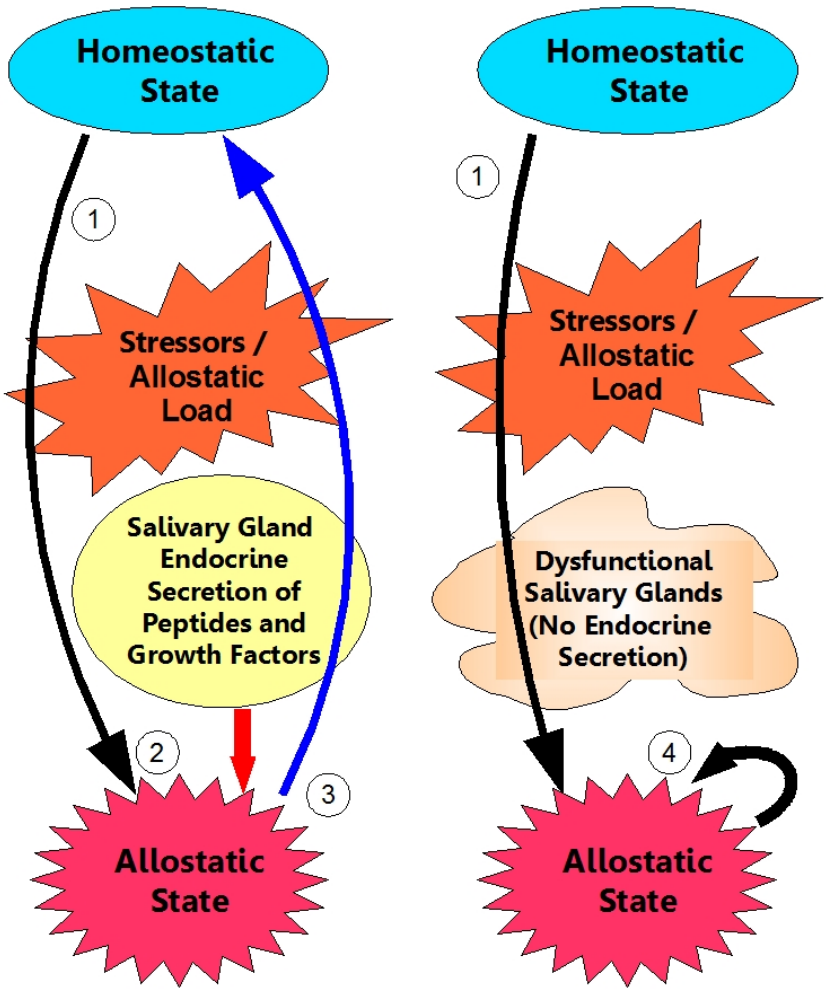

Fig. (4). Salivary Glands and Allostasis. Left side of figure: Stressors or allostatic load (1) lead to altered state with less than optimal homeostasis – the allostatic state. Endocrine secretion of submandibular gland factors (2) modulates the reactivity of system, and help re-establish the homeostatic state (3). Right side of figure:. However, if endocrine secretion is reduced from salivary glands the appropriate adjustments required to re-establish the homeostatic state cannot be made and the allostatic state persists (4) with pathological consequences.

extensive. Testosterone, produced by the testis, increases the severity of mouth and stomach ulceration in both sham-operated and sialoadenectomized rats [150]. Pinealectomy augments water immersion and restraint stress-induced ulcerous lesions, and these lesions exhibit a circadian rhythm with an increase in the day and attenuation at night, reflecting diurnal changes in melatonin synthesis [151]. Integrative input from the central nervous system is also involved. Exposing rats to either chronic constant light or repeated immobilization stress, respectively, either inhibits [152] or activates [153] the neural sympathetic component to the salivary glands. The sympathetic innervation of the salivary glands also modulates systemic responses such as mast cell mediated TNFα-dependent cytotoxicity [154], acute hypotensive responses to endotoxin [140], and pulmonary inflammation following antigen challenge [85], probably through the regulation of endocrine secretion of factors that allow adjustment to allostatic load.

Further studies and evaluations from the perspective of the concept of allostatic load will determine the validity of this hypothesis, and possibly contribute to understanding the role of salivary glands in modulation of systemic disease and pathology.

ABBREVIATIONS

ARDS	=	Acute respiratory distress syndrome
ALS	=	Amyotrophic lateral sclerosis
AMP	=	Adenosine 5'-monophosphate
bFGF	=	Basic fibroblast growth factor
BHR	=	Bronchial hyperresponsiveness
BNDF	=	Brain-derived neurotrophic factor
CD16	=	Low affinity FcγRIII receptor
EGF	=	Epidermal growth factor
E-FABP	=	Epidermal fatty acid binding protein
GVHD	=	Graft-versus-host disease
HGF	=	Hepatocyte growth factor
HPA	=	Hypothalamo–pituitary–adrenal
hHSP60	=	Human heat-shock protein 60
HIV	=	Human immunodeficiency virus
ICAM-1	=	Inter-cellular adhesion molecule 1

IGF-I & IGFII	=	Insulin-like growth factors I and II
LPS	=	Lipopolysaccharide
MPO	=	Myeloperoxidase
MHC	=	Major histocompatibility complex
mAChRs	=	Muscarinic acetylcholine receptors
NGF	=	Nerve growth factor
NOD	=	Non-obese diabetic
NSAIDs	=	Nonsteroidal anti-inflammatory drugs
PAM	=	Peptidylglycine alpha-amidating mono-oxygenase
PDGF	=	Platelet derived growth factor
PKC	=	Protein kinase C
SS	=	Sjögren's syndrome
SGPT	=	Submandibular gland peptide-T
SIRS	=	Systemic inflammatory response syndrome
SMR1	=	Submandibular rat-1
TGFα & TGFβ	=	Transforming growth factor alpha & beta
VEGF	=	Vascular endothelial growth factor
VIP	=	Intestinal peptide (VIP)
VLA4	=	σ4β1 integrin or CD49d

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