THE FUNCTION OF SALIVARY PROTEINS AND THE REGULATION OF THEIR SECRETION BY SALIVARY GLANDS

Gordon B. Proctor and Guy H. Carpenter
Secretory and Soft Tissue Research Unit, King's College School of Medicine and Dentistry, London, UK

SUMMARY

Salivary glycoproteins give saliva its characteristic physical properties and enable it to form a thin film over hard and soft tissues in the mouth. Oral health and homeostasis are dependent upon the functions performed by the salivary film and most of these functions, including lubrication, barrier function and microbial interactions, are in turn dependent upon salivary proteins. Some salivary proteins appear to fulfil more than one function and some functions are performed by a number of different proteins. There are relatively great variations in amounts of different proteins present in salivas from different subjects. However, subjects with low levels of particular proteins do not appear to suffer terms of oral health and this may be due to functional compensation by other proteins. Salivary protein secretion by salivary glands is dependent upon stimuli mediated by sympathetic and parasympathetic nerves and both acinar and ductal cells make a contribution to protein secretion. In addition to the well-characterized storage granule exocytosis pathway of protein secretion, salivary cells can secrete proteins by vesicular, non-storage granule pathways. These include direct secretion of newly synthesized proteins to saliva and to the glandular matrix and to circulation, and transcytosis of polymeric immunoglobulin A into saliva following secretion by glandular plasma cells. Recent data indicate that all of these pathways are subject to regulation by autonomic nerves. Resynthesis of some salivary proteins following secretion also shows a dependency upon nerve-mediated stimuli. The distal intracellular mechanisms coupling stimulation to synthesis are uncertain although the proximal events appear to be similar to those coupling stimulation to exocytosis. The synthesis of some salivary proteins can be upregulated by cytokines released from inflammatory cells and this can lead to increased salivary levels of antimicrobial proteins including lactoferrin and immunoglobulin A.

INTRODUCTION

The importance of salivary oral health is best illustrated in those who have chronic xerostomia. They experience difficulty in eating and swallowing and even speaking and may experience a bad taste, 'burning' mucosa, widespread mucosal and carious lesions associated with candidal and bacterial infection (1). Saliva performs a number of functions which are crucial to the maintenance of oral homeostasis. Some of these functions such as the moistening of food before swallowing or the removal of food residues and debris from the mouth could in theory be fulfilled by the presence of water or any other fluid in the mouth. However, saliva has special physical and biochemical properties which result from its composition and enable it to fulfil a number of other functions. Most of these functions are dependent to a large extent upon the protein components of saliva.

In this review we shall describe some of the structural features of salivary proteins associated with these functions. Whole mouth saliva is made up of the contributions from the parotid,
submandibular and sublingual and minor salivary glands and salivary proteins secreted by cells present in these different glands. Clearly if oral health is dependent upon salivary proteins then it is also dependent upon the mechanisms which control the synthesis and release of salivary proteins. In the second part of this review we will describe aspects of the control exerted over salivary protein secretion by nerves.

SALIVARY FILMS AND PROTEIN PELLICLES

- The sliminess of whole mouth saliva is a defining characteristic which we all become familiar with even from a very young age. This quality is imparted by the glycoproteins present in saliva, in particular by the two salivary mucins MG1 and MG2 (2). MG1 is typical of mucins found on other mucosal surfaces as it has a high molecular weight mucin (> 1000kD), is heavily O-glycosylated and has a strong negative charge due to the presence of terminal sulphation and sialylation on these O-linked sugar chains (2). MG2 is also heavily O-glycosylated but unusually for a mucin, has a relative molecular weight of approximately 100kD with little terminal sulphation. The salivary mucins are secreted by the minor salivary glands, palatal and labial, but mostly by the submandibular glands (SMG) and sublingual glands. Comparison of the mucins secreted by individual glands reveals that they have same peptide structures but some differences in posttranslational glycosylation (3). The viscoelasticity of mucins is a direct result of their molecular structure as the abundant O-linked sugars, in particular the N-acetylglucosamine residues linked to the serine and threonine residues, impose an extended 'bottle-brush' conformation, the sugar chains being the bristles. Owing to the presence of naked, hydrophobic regions and cysteine residues, a tertiary, cross-linked structure can form which effectively increases molecular weight (2,3). Under resting conditions, that is in the absence of overt stimulation of salivary flow, the volume of saliva in the mouth is only approx. 0.8 ml and this small volume is distributed as a slow-moving thin layer (0.8 mm/mur) over the hard and soft tissues of the mouth (4). The mucins and the properties that they impart to saliva appear to be crucial to the presence of a moisture retentive barrier of high film strength at the interface of soft tissues and the outer environment. This barrier is fundamental to the protection of the sensitive oral mucosa as it prevents dessication, can reduce permeability to potential toxins and lubricates thus preventing physical damage. The mucosal barrier is based upon MG1 and MG2 but also contains the other functionally important salivary proteins. These include secretory immunoglobulin A (sIgA), the principle mucosal immunoglobulin, various proline-rich proteins.

![Salivary film](image)

**Figure 1.** Salivary films. Saliva forms a film over the hard and soft tissues of the mouth and most of the important properties of this film are dependent upon the salivary proteins. In addition salivary proteins form an adherent layer on teeth, referred to as the acquired enamel pellicle, which reduces demineralization and lubricates.
Function and regulation of salivary gland secretion

(MGP), amylase, cystatins and others. Mucins may form noncovalent heterotypic complexes with some of the other salivary proteins to further improve their properties (5,6). The function of such complexes is likely to vary according to the protein involved. The association of MGI with statherin, for example, enhances lubrication by saliva whilst the interaction of PRP with MGI might provide a repository for precursors of the acquired enamel pellicle (6). Given that the unstimulated salivary film is slow-moving it is likely that its protein composition varies on different oral surfaces depending upon their proximity to different glandular secretions. Mucins are all but absent from parotid saliva. Nevertheless, heterotypic complexes of non-mucinous salivary glycoproteins can occur in parotid saliva (5) and it may be that these fulfill tissue coating functions similar to those found in mucin-containing salivas. It is likely that saliva also forms a film over teeth although it is uncertain how the dynamics and thickness of such a film compare with that on soft tissues. In addition to such a mobile film, the enamel surface of teeth is covered by an adherent layer of salivary proteins referred to as the acquired enamel pellicle (7) (Fig. 1, Table 1). Various salivary proteins have been found in the pellicle including MGI (8), acidic PRP (9), and cystatins (8). The mechanism(s) by which these proteins adhere is not known although in the case of acidic PRP it is likely to be through charge interaction of phosphorylated serines with hydroxyapatite. The acquired enamel protein pellicle appears to act as a lubricant reducing occlusal wear and as a barrier to demineralization.

VARIATIONS IN SALIVARY PROTEIN COMPOSITION AND FUNCTION

- In cross-sectional studies of human salivary proteins it quickly becomes apparent that there is a high degree of variation between individuals in the amounts of different proteins. Such variation is well-demonstrated by SDS PAGE of parotid salivary proteins and is most apparent in PRP (10,11). These are proteins which are peculiar to saliva and are particularly prominent in parotid saliva where they make up to 80% of total salivary protein (12). The high degree of genetic polymorphism in these proteins has been shown (13). PRP can be divided into two groups on the basis of their pi: basic PRP have a high pi and acidic PRP a low pi (10). Acidic PRP, by virtue of the PO₄³⁻ groups present on the N-terminal serine residues have been shown to bind Ca²⁺. As well as binding to the enamel surface they play an important role in maintaining saturated levels of Ca²⁺ in saliva (14). The function of the basic PRP is less certain but may include aggregation of oral bacteria and binding of dietary tannins which have been shown to have detrimental effects in animal studies (15). Apart from making cross-sectional studies of different patient groups difficult, this high degree of inter-individual variation in PRP and other salivary proteins indicates that they have overlapping function (16). As salivary proteins have been purified and investigated it has become apparent that different salivary proteins can fulfill the same function. For example, statherins fulfill a similar role to acidic PRP in Ca⁴⁺ homeostasis and tooth mineralization whilst another group of proteins, the histatins, have been found to bind dietary tannins even more strongly than PRP (17). Allied to this functional overlap individual salivary proteins can fulfill a number of different roles (16). Thus statherins function not only in oral Ca⁴⁺ homeostasis but also in boundary lubrication (18), whilst mucins are important in tissue coating and can bind oral bacteria (2).

**INTERACTIONS OF SALIVARY GLYCOPROTEINS WITH BACTERIA**

- There are a number of mechanisms by which viral, fungal and bacterial colonization of hard and soft tissues in the mouth is prevented. With the exception of desquamation of mucosal epithelial cells these mechanisms are all dependent on saliva and with exception of the physical movement of saliva around the mouth, which provides a general cleansing, these are all dependent upon salivary proteins. Increasingly, data is being generated on antiviral salivary proteins. Examples of such proteins are the cystatins, one of which, cystatin C, has been found to block replication of *Herpes simplex* virus (19); slgA and mucins interact with influenza virus via sugar residues, a mechanism similar to that described below for bacteria (20); and leukocyte secretory protease inhibitor, which has anti-HIV 1 activity (21). The histatins, a group of cationic histidine-rich

**Table 1. Salivary proteins are multifunctional**

<table>
<thead>
<tr>
<th>Function</th>
<th>Salivary protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibacterial</td>
<td>Amylase, immunoglobulin A (IgA), lactoferrin, lysozyme, mucins, peroxidase, histatins, cystatins, proline-rich proteins (PRP)</td>
</tr>
<tr>
<td>Antifungal</td>
<td>Histatins</td>
</tr>
<tr>
<td>Antiviral</td>
<td>IgA, mucins</td>
</tr>
<tr>
<td>Eubrication</td>
<td>Mucins, statherins</td>
</tr>
<tr>
<td>Mineralization</td>
<td>PRP, statherins</td>
</tr>
<tr>
<td>Tissue coating</td>
<td>Amylase, mucins, PRP, statherins</td>
</tr>
</tbody>
</table>

Biomell Rev 9, 1998
probes, revealed that many other major parotid salivary proteins are glycosylated (11). Exocrine glands with serous cell types such as the parotid gland, have been thought to only N-glycosylate proteins and so a further novel finding of the latter study was that many parotid salivary proteins were O-glycosylated. In particular, the lectin binding and specific glycosidase digestions performed indicated the presence of the same sugar sequence found to be important in mediating the interaction of mucins with oral streptococci, that is sialylated galactose-CD,3-N-acetylgalactosamine (27). The presence of this sugar may account for the observed interaction between GI and other parotid proteins with certain oral bacterial species including oral streptococci (25,28,29).

There are aspects of the interactions between salivary glycoproteins and oral bacteria which are disadvantageous to the host. The presence of glycoproteins, particularly MG1 in the acquired enamel pellicle provides bacterial binding sites and therefore favours the attachment of particular oral bacterial species which are the first wave in the formation of plaque and have a cariogenic effect (8). In fact virtually all surfaces are susceptible to bacterial colonization (30). It seems that bacterial plaque and its associated problems are a necessary evil off-set by the paramount requirement for a renewable protein pellicle on teeth which prevents the wearing down of a non-renewable enamel surface. Glycoproteins can serve as a source of nutrients to those oral bacteria species which have the glycosidase enzymes capable of digesting the terminal sialic acids and neutral sugars present on salivary glycoproteins. Again, much of the evidence for the latter has been gained from in vitro studies which suggest that bacterial species can act ‘cooperatively’ in utilizing glycoproteins as substrates (31).

SECRETION OF SALIVARY PROTEINS

- In contrast with studies of the structure and function of salivary proteins which have mostly been conducted on readily available human samples, studies of the control of salivary secretion have mostly been conducted in animal models. Salivary secretion of fluid and proteins is regulated by efferent parasympathetic and sympathetic autonomic nerves that innervate salivary glands and once these nerves have been sectioned secretion ceases almost entirely (Fig. 2). A minority of salivary glands are additionally capable of secreting saliva in the absence of impulses from nerves, a phenomenon referred to as spontaneous secretion (32). The pattern of innervation of different salivary glands within and between species varies greatly, particularly with respect to the sympathetic innervation and this is reflected in the different fluid and protein secretory responses that can be obtained by electrically stimulating these nerves (33). The main protein-secreting cells in salivary and other exocrine glands are the acinar cells which contain large numbers of protein storage granules. These cells have been the focus of research into salivary protein secretion. In many salivary glands,
Function and regulation of salivary gland secretion

Figure 2. Control of salivary secretion by nerves. Parasympathetic and sympathetic autonomic nerves are the efferent arms of the salivary taste and chewing reflexes and control fluid and protein secretion by salivary cells. The only nerve-mediated inhibitory influence on salivary secretion is from the higher centres of the brain under conditions of stress or anxiety.

significantly the rat parotid and submandibular glands, in which protein secretion has been most extensively studied, the sympathetic nerves appear to provide the main impetus for salivary protein secretion. Stimulation of the sympathetic nerves leads to a profound exocytosis of storage granules from the protein storing acinar cells and secretion of saliva rich in protein. The sympathetic stimuli evoking exocytosis of storage granules are mediated by (3-adrenoceptors on acinar cells and intracellular coupling of stimulus to secretion involves rises in cAMP and the activity of protein kinase A (34,3 5). Stimulation of the para-sympathetic nerves in general leads to secretion of a copious saliva containing lower concentrations of protein (36). These para-sympathetic stimuli are mediated through muscarinic cholinergic receptors (34). During feeding, both sympathetic and para-sympathetic nerves mediate taste and chewing stimuli and the saliva formed does not exhibit the contrasting features of the salivas secreted upon stimulation of individual nerve supplies. When the parasympathetic and sympathetic nerves are electrically stimulated simultaneously under experimental conditions, in an attempt to more closely approximate events in life, there tends to be an augmented secretion of protein, that is, protein output is greater than on individual nerve stimulation, reflecting that the nerves tend to cooperate rather than antagonize each other's secretory effects (37). Ductal cells have a well-recognised role in modulating the ionic composition of saliva but are also able to secrete proteins. In man and cat, the proteolytic enzyme kallikrein has been localized in small apical secretory granules of ductal cells (38) whilst in rats and mice the ductal cells have developed into major protein storing cells, the granular duct cells (39). In all of these ductal cells sympathetic nerve stimuli again provide the main impetus for protein secretion except this time mediated mainly through a-adrenoceptors whilst parasympathetic nerves again appear to have little effect (40-42).

The Nobel prize winning studies of Palade and coworkers in the pancreatic acinar cell traced the pathway taken by secretory protein following synthesis and incorporation of radiolabelled leucine (43). In similar studies on the rabbit parotid acinar cells the time taken for radiolabelled protein to be exocytosed from storage granules across the apical plasma membrane following synthesis was at least 3.5 hrs (pathway 1, Fig. 3; 44). Radiolabelled proteins progressed rapidly through the rough endoplasmic reticulum, Golgi complex and spent most time within the maturing storage granule compartment before exocytosis. This mechanism accounts for the bulk of protein secretion from the salivary glands and all of the major salivary proteins appear to be secreted in this way by acinar cells. Thus, it has been found that, regardless of the autonomic protein secretory stimulus applied, the proportions of major proteins secreted by salivary glands were not grossly different (40). Unfortunately this led to an acceptance by most researchers of exocytosis of storage granules as the exclusive mechanism of protein secretion by salivary and other exocrine cells.
SECRETION OF SALIVARY PROTEINS BY OTHER ROUTES

- Studies of protein transport in pituitary tumor (AtT-20) cells, a cell type that stores secretory proteins, led to the proposal that direct vesicular transport could take place in all cells, even endocrine, exocrine and nerve cells that secrete by regulated storage granule exocytosis. The pathway was termed a constitutive pathway to indicate that proteins were secreted as fast as they were synthesized (45) (pathway 3, Fig. 3). Evidence for the existence of non-storage granule secretory pathways in exocrine acinar cells was obtained in radiolabelling studies performed on parotid and pancreatic tissue in vitro which revealed that there is a release of newly synthesized protein (46). At approximately 40 min following radiolabelling a small, up to 15% of total, release of radiolabelled protein occurred whilst the main peak of secreted radiolabelled proteins characteristic of the regulated storage granule pathway, occurred from 3.5 hr onwards. The kinetics of the first secretory episode were not characteristic of direct vesicular trafficking from Golgi complex to plasma membrane but occurred when.

**Figure 3.** Protein secretory pathways from salivary cells. The great majority of salivary protein is secreted by the storage granule/exocytosis pathway (1) and degradation is activated primarily by stimuli from sympathetic nerves. In the constitutive-like pathway (2) proteins are secreted into saliva in vesicles which bud from immature storage granules whilst in the constitutive pathway (vesicles carry protein directly to the apical (3) or basolateral (4) cell surfaces from the Golgi complex. The transcytosis of polymeric immunoglobulin A (plgA) from the basolateral to apical membrane (5) is dependent upon membrane-bound polymeric IgA receptor (plgR).
radiolabelled protein was present in immature secretory granules and further evidence lead the authors to conclude that it represented vesicular budding from immature granules. The pathway was referred to as constitutive-like (pathway 2, Fig. 3), to distinguish it from the direct constitutive pathway. It was always conceived that upregulation of constitutive vesicular secretion could occur indirectly through upregulation of protein synthesis. However, recent studies suggest that non-storage granule pathways are also subject to direct regulation (47). Low doses of the autono-mimetics caused selective discharge of newly synthesized proteins in the same proportions as seen in the constitutive-like pathway. Previously, studies of parotid protein secretion following electrical stimulation of autonomic nerves indicated that sympathetic nerve impulses provide the main impetus for storage granule exocytosis (36). Nevertheless protein secretion occurred on parasympathetic nerve stimulation in the absence of morphological evidence of degranulation (48). It appeared that parasympathetic nerve stimulation evoked amylase secretion by a non-storage granule pathway which was replenished by immediate resynthesis of protein. Injection of radiolabelled leucine followed by electrical stimulation of the parasympathetic auriculo-temporal nerve supply revealed a peak of radiolabelled protein secretion with very similar kinetics to the constitutive-like pathway, whilst during sympathetic nerve stimulation secretion of radiolabelled protein peaked at a much later time point (49).

Non-storage granule secretory routes have also been found to operate in salivary gland ductal cells. The granular duct cells of mice and rats secrete large amounts of tissue kallikreins, which are trypsin-like enzymes of restricted and defined substrate specificity (50) and in addition the mouse granular duct cells secrete renin, a vasoactive aspartic protease. Stored renin is secreted as a two-chain form upon stimulation. However, radiolabelling studies indicated that a one-chain form was secreted by a non-storage granule route (51). Sympathetic nerve stimulation of rat granular duct cells evokes a large secretion of tissue kallikreins associated with degranulation whilst parasympathetic nerve stimulation causes a secretion of 100 fold less enzyme with no evidence of storage granule exocytosis (42). Different proportions of the tissue kallikreins were present in parasympathetic saliva compared to sympathetic saliva and storage granules, as represented by a glandular homogenate (52,53). This suggested that a different secretory route which by-passes storage granules was responsible for the secretion of the small amounts of enzyme present in parasympathetic saliva. The proportions of the tissue kallikreins in parasympathetic saliva were very similar to those in glandular homogenates during the early phase of re-synthesis following an almost total degranulation induced by the autonomic mimetic cyclohexylamine (54). This evidence suggested that newly synthesized kallikreins were appearing in saliva during parasympathetic nerve stimulation. Demonstration that such stimulated non-storage granule secretion was related to a

constitutive secretory pathway was obtained by sampling kallikreins secreted by unstimulated glands between periods of parasympathetic nerve stimulation. The secreted kallikreins accumulated in lumina of the gland and the composition of these enzymes was the same as observed in parasympathetically evoked saliva (55). The functional importance of the non-storage granule secretory pathway is uncertain as all salivary secretory proteins appear to be represented to varying extents in all secretory pathways. However, given the differing proteins present in apical compared to basolateral membranes of salivary cells, particularly with regard to ion transporting proteins, it seems likely that Golgi-derived vesicles containing different membrane proteins are targeted (56). If vesicles are moving directly to the basolateral as well as the apical plasma membrane, do they deliver secretory proteins into the glandular interstitium and blood? Small but significant increases in blood levels of parotid amylase and SMG kallikrein upon electrical stimulation of glandular nerve supplies in the rat seem to be via a vesicular mechanism as the increases did not reflect the large salivary outputs of these enzymes associated with sympathetically evoked storage granule exocytosis (57,58). Morphological evidence of a basolateral movement of tissue kallikrein-containing vesicles has been found in mouse granular duct cells (59). It may be that the delivery of secretory proteins to the glandular interstitium and blood does not in itself fulfil a purpose but is incidental to the delivery of membrane proteins (pathway 4, Fig.3).

Intracellular trafficking pathways are even more complex than described so far as vesicles also move from basolateral to apical membrane delivering polymeric Ig A across cells. Polymeric Ig A is the product of plasma cells within salivary glands and is secreted ab initio into the interstitial matrix of salivary glands in a complex with J (joining) chain (60) and then enters saliva as secretory IgA (slgA), a complex of plgA and the epithelial cell-derived polymeric IgA receptor (plgR) (pathway 5, Fig.3). This protein is expressed in a number of different secretory epithelia in the respiratory and intestinal tracts and its control has been studied partly because of its impact on mucosal adaptive immunity. Immediate stimulation of IgA transcytosis is observed in epithelial cell lines following phosphorylation of plgR by protein kinases A or C (61,62). These findings prompted a recent study of the influences of autonomic nerve stimulation on slgA secretion by the rat SMG as the above kinases are part of the intracellular mechanisms coupling nerve stimulation to salivary secretion (34,35). It was found that sympathetic nerve stimuli upregulated slgA secretion 6 fold above a basal rate whilst parasympathetic stimuli upregulated it 3 fold (63).

CONTROL OF SECRETORY PROTEIN SYNTHESIS BY NERVES

- Nerves are responsible for the secretion of protein from salivary glands and stores of secretory proteins must be
replenished, but how is the resynthesis of secretory proteins controlled? Secretory protein resynthesis is well demonstrated in the parotid gland as it shows a diurnal variation in the secretory protein content associated with the feeding cycle. Following protein secretion induced by feeding, a rapid fall in glandular content of secretory proteins was accompanied and followed by a period of resynthesis during which the proteins were replenished. Resynthesis is dependent upon neurally mediated stimuli as it is greatly reduced by feeding rats a liquid diet which abolishes much of the stimulation arising from mastication (64). Protein secretion in the submandibular and sublingual glands of the rat shows less dependence upon the feeding stimulus, nevertheless an increase in submandibular protein synthesis on feeding has been demonstrated although it is of a lesser magnitude than that observed in the parotid gland (65). Maintenance of rats on a liquid diet for 1-2 weeks caused an atrophy of the parotid glands which was associated with a general reduction in protein secretory capacity (66,67). Such experiments indicated that the synthesis of different salivary proteins has a varying dependency on neurally mediated stimuli as analysis of the protein components of autonomically-evoked parotid saliva demonstrated changes in the composition of secretory proteins (67,68). Thus the proportions of PRP and amylase were reduced whilst other proteins remained unchanged. The influence of individual branches of the autonomic innervation on salivary protein synthesis has been investigated through the use of selective denervations followed by analysis of salivary protein composition. Proctor et al (69) performed unilateral sympathectomies on adult rats by removing the superior cervical ganglion and one week later obtained salivas from denervated and control contralateral glands by parasympathetic nerve stimulation. During such short-term sympathetic-nomy no significant glandular atrophy took place, nevertheless there was a profound change in the protein composition of saliva indicative of reduced synthesis of secretory proteins. In particular there were greater reductions in the content of PRP as a proportion of total protein (69). Similar changes in composition of secretory proteins were observed subsequently in glandular homogenates one week following sympathectomy (70) and in salivas obtained from chronically sympathectomized rats (71). Overall the results indicate that the synthesis rates of different parotid secretory proteins show differing dependencies on impulses arriving from sympathetic nerves. Similar changes were observed when rats were treated for 10 days with the (3-adrenoceptor blockers metaprolo l or propranolol (72). Parasympathetic denervation also causes changes in the synthesis of secretory proteins. In the cat SMG, it leads to a disappearance of stored tissue kallikrein in striated ductal cells (73) which is accompanied by massive reductions in the tissue kallikrein content of sympathetically-evoked saliva (74). This reduction in the salivary content of tissue kallikrein was seen following chronic muscarinic receptor blockade (75), so it would appear that synthesis of the enzyme is dependent specifically on stimuli mediated by acetylcholine. Short-term parasympathectomy of the rat parotid gland produced changes in the protein composition of sympathetically-evoked saliva with decreases in amylase content and levels of specific basic PRP (76). Whether the synthesis of these proteins was dependent specifically on acetylcholine or on one of the peptide cotransmitters present in parasympathetic nerves supplying salivary glands remains uncertain. The effects of nerve-mediated stimuli on rat parotid secretory protein synthesis were examined more directly by Asking and Gjorstrup (48) who measured the incorporation of radiolabelled leucine into proteins during electrical stimulation of the sympathetic or parasympathetic or both nerve supplies, in anaesthetized rats. Both parasympathetic and sympathetic nerve impulses doubled the incorporation of radiolabelled amino acid compared to contralateral unstimulated parotid glands and there was a much greater incorporation, indicative of augmented protein synthesis, when both nerves were electrically stimulated simultaneously (48). The receptor-mediated intracellular coupling mechanisms through which autonomic nerves exert these effects have been examined in vitro. Parotid protein synthesis is increased in response to a-adrenergic agonists and this effect appears to be mediated through increases in levels of intracellular cAMP (77). Similar results have been observed in dispersed submandibular acinar cells (78,79), oc-adrenergic agonists and cholinomimetics have been found to inhibit parotid and SMG secretory protein synthesis, apparently through increases in levels of intracellular calcium as the effect was mimicked by the calcium ionophore A23187 (78-80). However, lower doses of cholinergic agonists, 0.1 uM rather than 10uM carbachol, caused increases in SMG protein synthesis (79). The latter result coincides with the increased synthesis observed on parasympathetic nerve stimulation of the parotid gland (48) and suggests that it too involves acetylcholine, possibly acting with concomittantly released peptide neurotransmitters.

The distal intracellular mechanisms activated by rises in the intracellular messengers cAMP and calcium which lead to changes in rates of protein synthesis are at present uncertain. Likewise it is unclear whether nerve-mediated stimuli induce changes in rates of translation, transcription or both. A consistent observation in protein radiolabelling studies following feeding or stimulation with sympathomimetics, in vitro or in vivo, has been that maximal rates of protein synthesis occur approximately 6 h following the stimulus (80, 81). It appears that this delay is due in part to upregulation of mRNA levels for secretory proteins through cAMP-mediated protein phosphorylation (82); possibly through the protein products of proto-oncogenes such as c-fos which are also upregulated as a result of a-adrenergic stimulation and may play a role in the regulation of the other inducible genes, although such a role in salivary glands has yet to be established (83). Repeated pharmacological doses of iso-prenaline, as well as causing rat parotid and submandibular
gland enlargement, induce a massive synthesis of PRP. This effect is mediated by cAMP and elevations in levels of mRNA $^{PRP}$ (84). The upstream regions of the mouse and hamster PRP genes contain putative regulatory sequences for cAMP induction (84) and removal of these sequences prevented the isoprenaline-induced PRP synthesis (85). As such sequences are absent from a characterized human gene (86) it may be that the synthesis of human PRP is not dependent on p-mediated stimuli. Recent results in which incorporation of radiolabelled proline into separate PRP and non-PRP fractions of glandular homogenates was measured in sympathetically and double denervated parotid and submandibular glands suggest that parasympathetic and sympathetic nerves are important for maintaining the synthesis of mRNA $^{PRP}$ in both glands. The effect of double denervation represented the additive effects of the individual denervations (87,88).

Increases in transcriptional rates and delayed upregulation of protein synthesis do not account for all nerve-mediated increases in secretory protein synthesis. In many of the reported studies incorporation of radiolabelled amino acid was increased within 1 hr of commencing stimulation. Such early changes suggest that substantial amounts of mRNA for secretory proteins were already present in cells which had previously been quiescent. This suggests that protein synthesis is also upregulated by a translational mechanism as, originally proposed by Grand and Gross (89). A recent in vitro study on parotid acinar cells suggested that higher doses of cholinergic agonists (10 μM carbachol) cause early reductions in amylase synthesis by reducing translation and destabilizing mRNA (80). The effects of calcium mobilizing agonists on salivary protein synthesis seem paradoxical given that protein synthesis is dependent upon phosphorylation of a number of translation initiation factors, eIF-2B, eIF-3 and others, which are the targets of calcium and diacylglycerol-dependent protein kinase C (90).

Thus it appears that both transcriptional and translational control is exerted on salivary secretory protein synthesis in the rat. It may be that individual secretory proteins show different degrees of dependence on transcriptional control. Synthesis of PRP has a greater dependence on transcriptional mechanisms stimulated through α-adrenergic receptors and raised intracellular cAMP and this is demonstrated by the disproportionately greater changes in the levels of these proteins resulting from chronic treatment with α-adrenergic agonists or antagonists or as a result of denervation. In contrast, amylase synthesis may depend less on transcriptional control as suggested by the maintained levels of amylase mRNA in parotid cells of rats kept on a liquid diet which show greatly reduced levels of enzyme (91). Given these differences in the regulation of individual secretory proteins it would be interesting to determine how much the proportions of proteins differ in saliva collected from individuals on different days or weeks. Human parotid salivas appear not to show significant changes in protein composition over time (unpublished observations).

The use of single agonists in vitro has provided useful information on the mechanisms by which nerves might control protein synthesis. However, as with studies of protein secretion, it is apparent that the important effects of combined autonomic stimulation, which is likely to more closely approximate events in life, have been largely ignored. Thus it could be that the significant contribution of cholinergic stimuli to protein synthesis is not the inhibition seen at high doses of autonomic but rather stimulation at low doses most probably in combination with peptide and adrenergic agonists.

### THE EFFECTS OF INFLAMMATION ON SALIVARY PROTEIN SYNTHESIS AND RELEASE

- In episodes of inflammation a number of changes in salivary protein composition have been observed (92). Often these observations have been made in chronic inflammation associated with Sjögren’s syndrome, an autoimmune exocrinopathy characterized by destruction of salivary and lacrimal glands (1). However, such changes are not specific to autoimmune disease and have been observed in other chronic inflammatory diseases, for example sialolithiasis. Increases in salivary lactoferrin have been observed in a number of studies and illustrates one of the mechanisms responsible for these changes.

  There are two possible sources of salivary lactoferrin: in the absence of disease it is synthesized and secreted by ductal cells and possibly acinar cells (93). During inflammation its levels in saliva can increase more than 10 fold and a possible non-salivary cell source of the increased lactoferrin is neutrophils as lactoferrin is a major component of specific granules. However, neutrophils are not a prominent infiltrating cell in chronic inflammation and a recent study demonstrated that raised salivary lactoferrin was fucosylated (95), the only molecular feature that was previously found to distinguish milk lactoferrin from neutrophil lactoferrin (96). What is the mechanism causing the increase in salivary gland lactoferrin? Lactoferrin appears to be one of a number of salivary epithelial cell proteins whose expression is upregulated during inflammation owing to the influence of cytokines from inflammatory cells. Thus it has been shown by immunocytochemistry of chronically inflamed salivary glands that not only is lactoferrin expression increased in epithelial cells but also are the membrane-bound major histocompatibility class (MHC) I, MHCII antigens, plgR (94) and the salivary levels of the released peptide product of MHC I, p2-microglobulin, is also increased (97). The cytokine interferon-γ is an inducer of MHC expression in epithelial cells and has been demonstrated along with the cytokines tumor necrosis factor-α and interferon-4, to increase plgR expression in epithelial cell lines following at least 12 hours exposure to the cytokines. This increase was found to be dependent upon protein synthesis as it could
be blocked with cycloheximide (98). It is likely that this cytokine induced increase in plgR expression represents a mechanism by which IgA delivery to mucosal surfaces can be maximized during mucosal infection. It may also be that the mechanism serves as a means by which IgA-antigen immune complexes can be excreted from the interstitial matrix and onto mucosal surfaces where they will be flushed-away (99).

REFERENCES


24. Ofek I, Perry A. Molecular basis of bacterial adherence to tissues. Ibid.


30. Beachey EH. Bacterial adherence: adhesin-receptor inter-


52. Proctor GB, Zhang XS, Garrett JR, Shori DK, Chan K-M. The enzymic potential of tissue kallikrein rK 1 in rat submandibular saliva depends on whether it was secreted via constitutive or regulated pathways. Exp Physiol 1997; 82:977-983.


61. Cardone MH, Smith BL, Mennitt PA, Mochly Rosen D, Silver RB, Mostov KE. Signal transduction by the po-
85. Wright PS, Carlson DM. Regulation of proline rich protein and a amylase genes in parotid hepatoma hybrid cells.
93. Korsrud FR, Brandtzæg P. Characterization of epithelial elements in human major salivary glands by functional markers: localization of amylase, lactoferrin, lysozyme, secreto-


