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

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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 vesi cular, 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. (**Biomed Rev** 1998; 9: 3-15)*

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INTRODUCTION

• The importance of salivate 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 fulfiled by the presence of water or any other fluid in the mouth. However, saliva has special physical and biochemical properti es which result from its composition and enable it to fulfil anumber 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 AMP PROTEIN PELLICLES

• The sliminess of whole mouth saliva is a defining cha racteristic which we all become familiar with even from a very young age. This quality is imparted by the glycoproteins pre sent 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 ahigh molecular weightmucin(> lOOOkD), 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 muc in, has a relative mo lecular weight of ap proximately 1001<D 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-acetylgalactosamine residues linked to the serine and threoni-ne 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[l]) 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 MG 1 and MG2 but also contains the other functionally important salivary proteins. These include secretory immunoglobulin A (slgA), the principle mucosal immunoglobulin, various proline-rich proteins

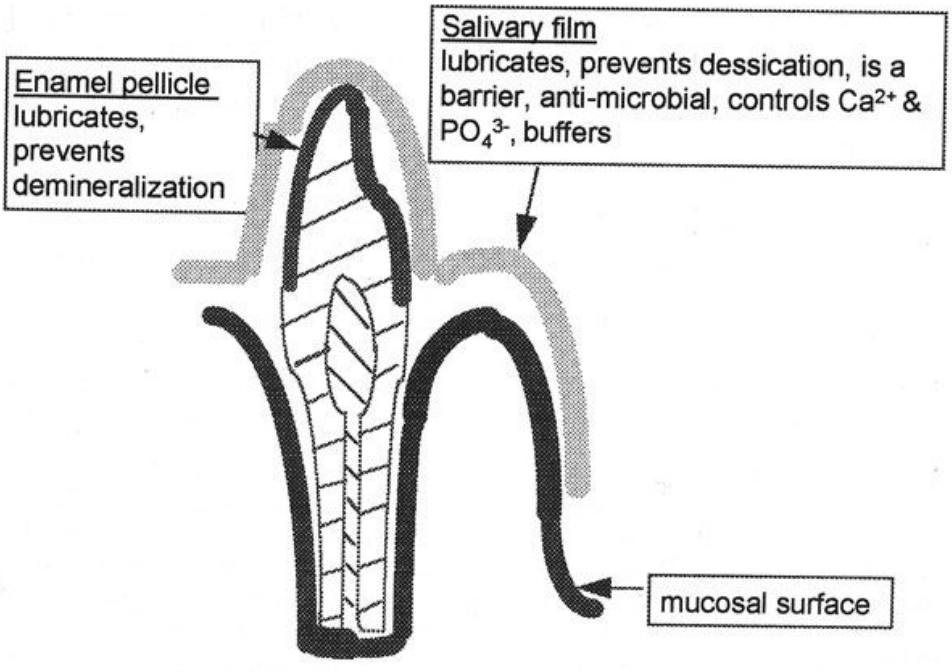

Figure 1. *Salivary films. Saliva forms a film over the hard and soft tissues of the mouth and most of the important properties of this film are dependent upon the salivary proteins. In addition salivary proteins form an adherent layer on teeth, referred to as the acquired enamel pellicle, which reduces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

(PRP), amylase, cystatins and others. Mucins may form noncovalent heterotypic complexes with some of the other salivary proteins to further imporve their properties (5,6). The function of such complexes is likely to vary according to the protein involved. The association of MG1 with statherin, for example, enhances lubrication by saliva whilst the interaction of PRP with MG1 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 fulfil 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 MG1 (8), acidic PRP (9), and cystatins (8). Themechanism(s) by which these proteins adhere is not known although in the case of the 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 varia tion 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 promi nent in parotid saliva where they make up to 80% of total sali vary 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 PO_4^{3}~ groups. present on the N-tenninal serine residues have been shown to bind Ca^{2+}. As well as binding to the enamel surface they play an important role in maintaining saturated levels of Ca^{2+} 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 fulfil the same function. For example, statherins fulfil a similar role to acidic PRP in Ca^{2+} 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 fulfil a number of different roles (16). Thus statherins function not only in oral Ca^{2+} 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, fun gal 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 the 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

Function	*Salivary protein*
Antibacterial	Amylase, immunoglobulin A (IgA), lactoferrin, lysozyme, mucins, peroxidase, histatins, cystatins, proline-rich proteins (PRP)
Antifungal	Histatins
Antiviral	IgA, mucins
Eubrication	Mucins, statherins
Mineralization	PRP, statherins
Tissue coating	Amylase, mucins, PRP, statherins

proteins, appearto have antifungal properties (22). However, far more data exists concerning the interactions of antibacterial salivary proteins and oral bacteria. The former form a broad range of proteins from lactoperoxidase, lysozyme and lactoferrin which attack bacterial cell walls (20), to glycoproteins such as mucins which interact with bacterial sugar receptors, as well as specific interactions between bacterial antigens and slgA (23). The significance of the salivary antibacterial proteins is disputed as most data has been generated from *in vitro* studies of purified proteins; there is relatively little direct *in vivo* evidence which conclusively proves that salivary proteins are effective in preventing bacterial infection. Thus the presence of the normal bacteria flora and dental plaque can be cited as evidence of the ineffectiveness of antibacterial proteins. However, the make-up of the bacterial species and the number of bacteria that colonize oral surfaces probably reflects the net influence of salivary protein interactions with bacteria (2). One way of obtaining *in vivo* evidence of the significance of antibacterial proteins is to examine conditions in which there is an absence of specific proteins. IgA deficiency is one of the few examples of such conditions, but there is little conclusive evidence of an increase in incidence of disease in the mouth (23). The presence of a range of antibacterial proteins may well partly explain the lack *of in vivo* data as again it is an example of the functional overlap referred to earlier, that is different proteins can fulfil the same function. Under conditions in which a range ofproteins are reduced as in xerostomia, then the effects on oral health are more severe. The sugar structures present on many salivary glycoproteins are at 'the front line' of salivary protein interactions with some bacteria and viruses. Many microorganisms have receptors, adhesins or lectins, which recognize and bind to specific sugar sequences found on mammalian cell surfaces and this forms a means by which microbial colonization of mucosal surfaces can occur (24). Those same sugar sequences are found on salivary glycoproteins and mediate their interaction with microorganisms. Such interactions are thought to act in two ways to benefit the host. Firstly, MG2 and other salivary glycoproteins can saturate potential mucosal binding sites on bacteria preventing them from binding to epithelial cells. Secondly, such interactions can also cause the aggregation of oral bacteria and such aggregates are thought to be less capable of mucosal colonization and more easily cleared from the oral cavity. The mucins, being the major glycoproteins of saliva, have been the focus of many descriptive studies of such interactions (2). Oral streptococci in particular have been found to interact with the smaller oral muc in MG2 and this interaction is mediated by sialylated or non-sialylated, depending on the species of streptococcus, galactose-al,3-N-acetylgalactos-amine structures in MG1 O-linkedsugarchains(3,25). In general, the major parotid salivary proteins have been considered to be unglycosylated with the exception of the basic PRP Gl (26). However, a recent study using sugar-specific labelled lectin probes, revealed that many other maj or 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 0-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 Gl 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 (3 0). 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 rea dily available human samples, studies of the control of salivary secretion have mostly been conducted in animal models. Saliva ry secretion of fluid and proteins is regulated by efferent para-sympathetic 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 absen ce of impulses fromnerves, aphenomenom referred to as spon taneous secretion (32). The pattern of innervation of different salivary glands within and between species varies greatly, parti cularly 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,

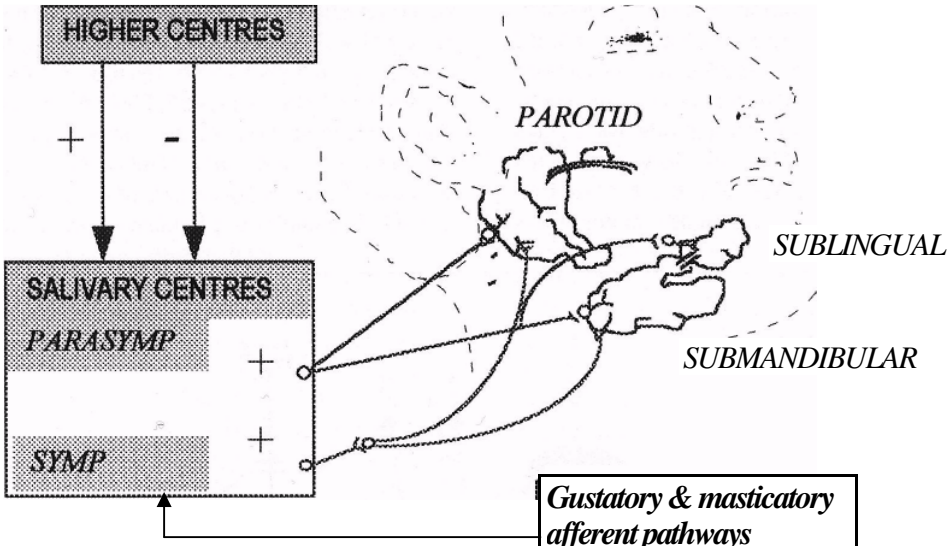

***Figure** 2. Control of salivary secretion by nerves. Parasympathetic and sympathetic antonomic nerves are the efferent arms of the salivary taste and chewing reflexes and control fluid and protein secretion by salivary cells. The only nerve-mediated inhibitory influence on salivary secretion is from 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 pa-rasympathetic stimuli are mediated through muscarinic choli-nergic receptors (34). During feeding, both sympathetic and pa-rasympathetic 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 endoplas-mic 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 autonomimetic 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 PROTEIMS 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). Evi dence 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

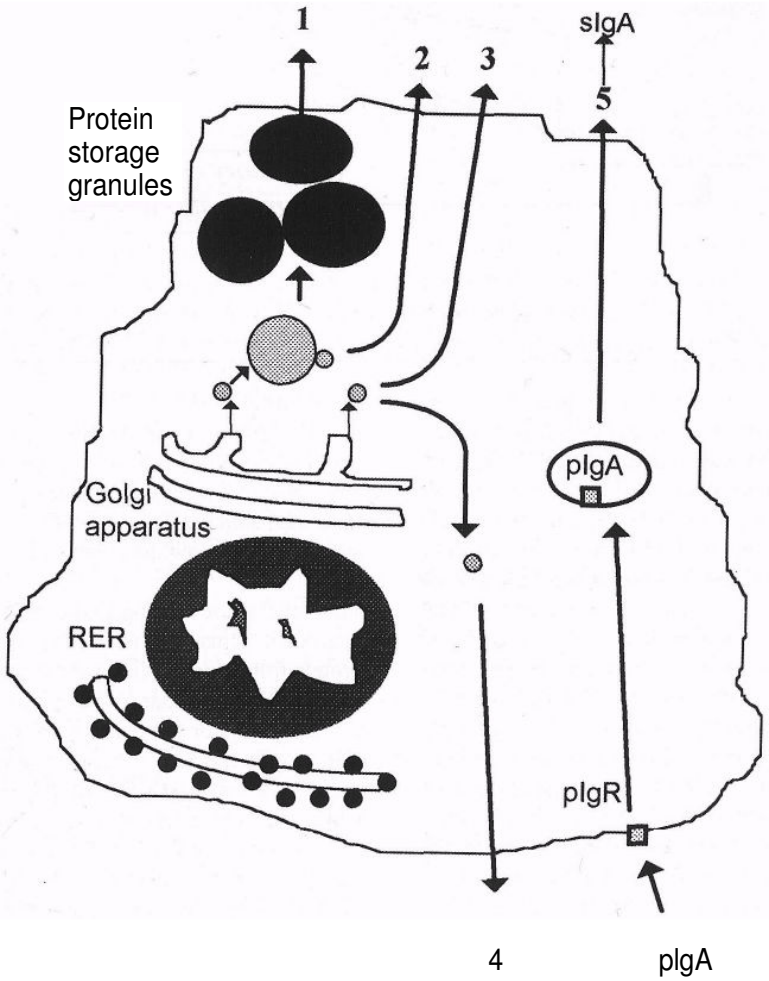

Figure 3. *Protein secretory pathways from salivary cells. The great majority of salivary protein is secreted by the storage gramile/exocytosis pathway (I) and degramtlation is activated primarily by stimuli from sympathetic nerves. In the constitutive-like pathway (2) proteins are secreted into saliva 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 immiinoglobulin 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). Differentproportions 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 similarto those in glandular homogenates during the early phase of re-synthesis following an almost total degranulation induced by the autonomimetic cylcocytidine (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 targetted (56). If vesicles are moving directly to the basolateral aswell 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 tissub 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 IgA 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 (sIgA), 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 IgAtranscytosis 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 sIgA 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 sIgA 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 weildemonstrat-ed 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 autonomimetically-evoked parotid salivas 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 innerva-tion 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 parasym-pathetic nerve stimulation. During such short-term sympathec-tomy 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 metaprolol 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 parasym-pathectomy 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 cotran-smitters 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-adren-ergic agonists and this effect appears to be mediated through increases in levels of intracellular cAMP (77). Similar results have been obtained 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 lOuM 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 hr 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 c AMP 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, parasympathetically and double denervated parotid and submandibular glands suggest that parasympathetic and sympathetic nerves are important for maintaining the synthesis of mRN A^{PRP} in both glands. The effect of double denervation represented the additive affects 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 mRN A 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 uM 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 cc-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 a-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 salivas 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 autonomimetic 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 autonomimetic 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 Sjugren's syndrome, an autoimmune exocrinopathy characterized by destruction of salivary and lacrimal glands (1). However, such changes are not specific to autoim mune disease and have been observed in other chronic inflam matory diseases, for example sialolithiasis. Increases in salivary lactoferrin have been observed in a number of studies and illu strates 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-saliva ry 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 infla mmation and a recent study demonstrated that raised salivary lactoferrin was fucosy lated (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 expre ssion 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 eel Is but so 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-y is an inducer of MHC expression in epithelial cells and has been demonstrated along with the cytokines tumor necrosis factor-a and interleukin-4, to increase plgR expression in epithelial cell lines follow ing at least 12 hours exposure to the cytokines. This increase was found to be dependent upon protein synthesis as it could

beblocked 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 ameans by which IgA-antigen immune complexes can be excreted from the interstitial matrix and onto mucosal surfaces where they will be flushed-away (99).

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