

NEURAL REGULATION OF THE PROTEIN COMPOSITION OF SALIVA

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SUMMARY

• *Fluid and protein secretion from salivary glands are primarily regulated by efferent parasympathetic and sympathetic nerves respectively. The protein concentration of saliva, which is made up of a number of functionally important proteins, can vary depending on the impulse traffic arriving from these nerves. The composition of the proteins secreted by a salivary gland with a mixed population of secretory cells can vary with different secretory stimuli and intensities of stimuli, according to how the individual cell populations respond. Most of the published evidence suggests that the protein composition of saliva secreted from the parotid gland, which has an apparently homogeneous secretory cell population, appears to be similar regardless of the type of nerve stimulus. It is likely that salivary secretory cells can secrete protein by a constitutive route, not involving storage granules, in addition to the regulated secretion of protein from storage granules, and that the constitutive route may operate during parasympathetic nerve stimulation. However the functional significance of this type of protein secretion is as yet unknown.*

In the longer-term the composition of proteins secreted from salivary glands also depends upon the rates of synthesis of individual proteins. Levels of secretory proteins show differing dependencies on specific nerve-mediated stimuli as shown by the changing compositions of proteins secreted following denervation or chronic pharmacological blockade. The levels of secreted proteins appear to be regulated at both the

transcriptional and translational levels of protein synthesis. In rodent salivary glands there is a dependency of secretory proline-rich protein synthesis on β -adrenergically mediated stimuli which is dependent upon putative cAMP regulatory sequences of nucleotides in the gene.

Although N-linked glycosylation has been shown to be regulated by β -adrenergically mediated stimuli, it is not known if the composition of sugars on proteins varies with neural stimuli.

INTRODUCTION

• "There can be no doubt that the main control of salivary secretion is exerted by nerves, and the glands have a rich supply from both divisions of the autonomic nervous system. After section of the nerves, secretion ceases almost entirely.....". This quote from Emmelin (1) is based on an understanding, gained over many years, of the profound influence exerted by parasympathetic and sympathetic nerves on salivary gland secretion. Although it is important to realize that the pattern of innervation of different salivary glands within and between species varies greatly, a consensus view is that sympathetic nerves provide the main impetus for salivary protein secretion whilst parasympathetic nerves drive the secretion of fluid; and these nerves tend to interact and augment secretion (2).

Investigations of salivary protein secretion have tended to focus on amylase, a prominent salivary enzyme providing a convenient marker for physiological studies in man, rat and rabbit. However in recent years many other salivary proteins have been purified and characterized and their functional significance uncovered. Most of these are products of acinar cells, for example acidic and basic proline-rich proteins and glycoproteins (3), mucins (4) and peroxidase (5) whilst some are secreted by ductal cells, for example tissue kallikrein (6) and lysozyme (7). Many of these proteins are now known to fulfil important protective functions in the mouth, for example in maintaining calcium homeostasis and tooth integrity and in altering bacterial adherence to hard and soft tissues (8). It is therefore of interest to study how the protein composition of saliva is regulated, firstly for the possible health implications of such regulation; secondly, as salivary glands are accessible exocrine glands, studies of the protein composition of saliva can yield information on the cellular mechanisms by which exocrine secretory protein composition is controlled.

In the ensuing paragraphs I shall review some of the published evidence demonstrating that the protein composition of saliva is regulated by nerves. Most of the experimental studies referred to involved rats although some studies, particularly those involving reflex stimulation, were performed in other experimental animals and humans. For the purposes of this review the regulation of protein composition will be divided on a timescale into immediate and longer term effects. The reason for such a division follows from a consideration of the cell biology of exocrine protein secretion. Like other exocrine cells, secretory cells in salivary glands store large amounts of protein in granules. Such storage granules contain previously synthesized proteins which, following receipt by the cells of the necessary stimulus, are secreted (9, 10). The composition of proteins present in storage granules will generally reflect the recent history of the cells. Therefore the longer-term regulation of salivary protein composition may often reflect previous changes in rate of synthesis of different, now stored, proteins.

IMMEDIATE REGULATION BY NERVES OF SALIVARY PROTEIN COMPOSITION

The main physiological stimuli giving rise to nerve-mediated secretion of fluid and protein from salivary glands are mastication and taste (Fig. 1). Gjoerstrup (11) performed a series of studies, using the chronically cannulated parotid gland of the conscious rabbit, in which he analyzed the contributions of chewing and taste to secretion of fluid and amylase. He found that the saliva evoked

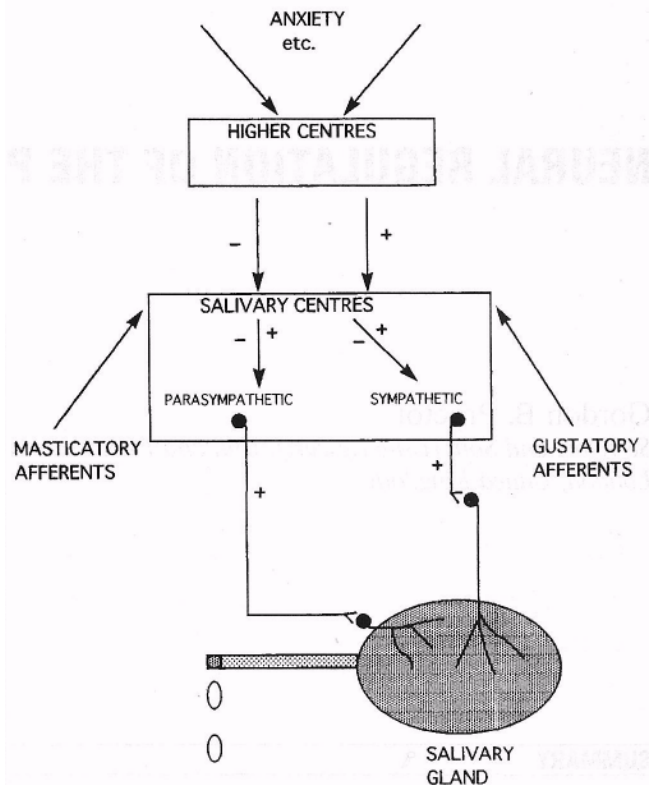


Figure 1.

Chart of nerve pathways involved in reflex secretion of saliva (modified from Garrett, 1987). + = facilitatory influences; - = inhibitory influences, that can act directly or indirectly on central salivary neurones. The parasympathetic and sympathetic nerves represent the efferent arms of the reflexes.

by feeding pellets contained a lower amylase concentration than saliva evoked by the sweeter carrot stimulus. The additional carrot-evoked protein secretion was mediated by sympathetic efferent nerves and was blocked by the (3-adrenoceptor antagonist propranolol. This work indicated that chewing is a parasympathetically-mediated stimulus for the secretion of fluid whilst sweet taste is a sympathetically mediated stimulus for the secretion of protein. Sweet taste alone, whilst evoking a secretion of protein, appears not to elicit a flow of saliva (12). Further work using the rabbit parotid model (13) indicated however that the total amount of protein and amylase secreted during chewing, because of the greater flow of fluid, was equal to or greater than that evoked by taste. Many studies of parotid protein and fluid secretion in response to different gustatory stimuli have been performed on human volunteers (e.g. 14,15,16). In general it was found that salt and sweet taste stimuli gave rise to salivas with higher protein and amylase concentrations than those evoked by a sour taste stimulus. Similar results to those of Gjoerstrup were

recently obtained in studies of human parotid secretion using mechanical and gustatory stimuli with and without (3-blockade (17). All of these studies of reflexly induced secretion indicate that different stimuli can give rise to salivas of differing protein concentration by changes in the ratios of parasympathetic and sympathetic efferent stimuli arriving at the gland. Less consideration was given to whether the composition of proteins secreted varies in such studies. Electrophoretic analyses of reflexly evoked human (15) and rabbit (13) salivas and measurements of amylase specific activities in human (18) and rabbit (13) salivas suggested that there were no gross differences in the protein compositions of salivas evoked by chewing or different gustatory stimuli.

Similar conclusions were reached following experimental studies using different efferent autonomic secretory stimuli of parotid glands in the anaesthetized rat (19,20,21). These investigations in general lend support to the viewpoint that proteins are secreted in a constant ratio regardless of the secretory stimulus, that is proteins are secreted in parallel. The parallel secretion of proteins by exocrine cells was thought by some authors to clearly establish that proteins are secreted only by the exocytosis of storage granules, a process first substantiated in the pancreas (8). It is now appreciated that at least some exocrine cells and maybe all, secrete proteins by exocytosis and by another more primitive mechanism, seen in many cell types and termed constitutive secretion (22). Constitutively secreted proteins are packaged in small vesicles budding off from the trans Golgi network and transported directly to the plasma membrane, by-passing the storage granule compartment (Fig. 2). Such secretion may help to explain some of the discrepancies observed in studies on exocrine protein secretion, particularly in the pancreas (e.g. 23,24), which did not fit into the concept of parallel secretion and which provoked a brisk debate. It has been demonstrated in the mouse submandibular gland that renin is secreted by a constitutive pathway in a different, newly synthesized, molecular form to that secreted by the regulated exocytotic pathway (25). The rate of constitutive secretion of renin appeared to be directly dependent upon the rate of renin synthesis. Constitutive secretion may explain the apparent lack of degranulation of rat parotid acinar cells following parasympathetically evoked protein secretion (10) in spite of the fact that under such conditions a substantial quantity of protein was released (26). From their radiolabelling studies Asking & Gjørstrup (26) proposed that during parasympathetic nerve stimulation amylase was synthesized and immediately secreted by a route not involving storage granules. However, as mentioned earlier, if proteins are secreted by a different route during parasympathetic compared to sympathetic stimulation of the rat

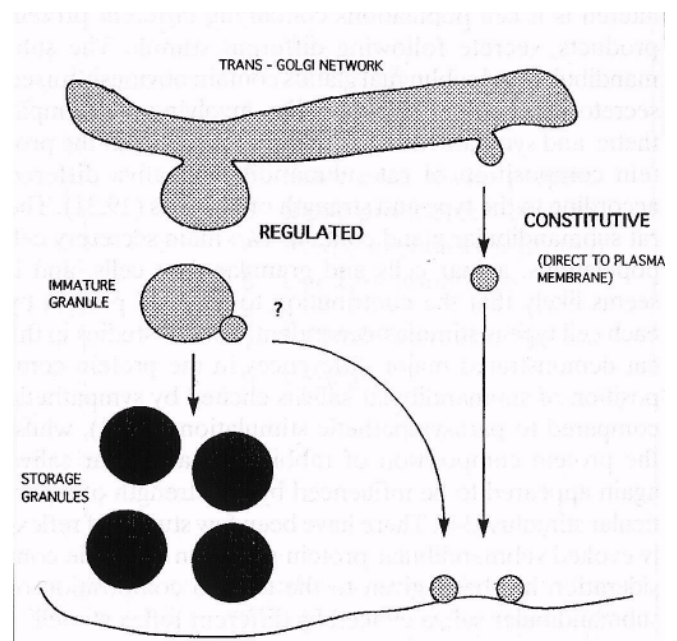


Figure 2.

An diagrammatic representation of the regulated and constitutive pathways of protein secretion. Large granules budding-off from the trans Golgi network (TGN) of the Golgi complex form mature storage granules through condensation of the secretory product; secretion of these awaits an appropriate stimulus. Smaller vesicles bud-off from the TGN, are transported directly to and fuse with the plasma membrane to release protein; this pathway forms the constitutive route. A constitutive-like pathway also operate when small vesicles bud-off from immature granules and fuse with the plasma membrane. The constitutive and constitutive-like routes by-pass the storage granule compartment.

parotid gland, this does not manifest itself in gross changes of protein composition, although observations of more minor differences have been made (20,27). Further evidence for the involvement of a different secretory route during parasympathetic compared to sympathetic stimulation was recently obtained in studies of proteinase secretion from the granular duct cells of rat submandibular glands (28,29,30,31). In these experiments obvious differences were seen in the composition of proteinases in the salivas elicited by different stimuli. Of particular interest was the finding that more glycosylated isoforms of tissue kallikrein were present in proportionately much higher amounts in parasympathetic saliva compared to sympathetic saliva. It appears therefore that salivary cells secrete by a non-regulated route, the functional significance of which is at present unknown.

Many of the studies cited above were performed on parotid glands which are generally thought to have a homogeneous population of secretory cells. An obvious way in which the protein composition of saliva secreted by a gland might be

altered is if cell populations containing different protein products, secrete following different stimuli. The submandibular and sublingual glands contain obviously mixed secretory cell populations. Studies involving parasympathetic and sympathetic stimuli demonstrated that the protein composition of rat submandibular saliva differed according to the type and strength of stimulus (19,31). The rat submandibular gland contains two main secretory cell populations, acinar cells and granular duct cells, and it seems likely that the contribution to secreted protein by each cell type is stimulus-dependent. Similar studies in the cat demonstrated major differences in the protein composition of submandibular salivas elicited by sympathetic compared to parasympathetic stimulation (32,33), whilst the protein composition of rabbit submandibular saliva again appeared to be influenced by the strength of a particular stimulus (34). There have been few studies of reflexly evoked submandibular protein secretion and little consideration has been given to the protein composition of submandibular saliva evoked by different reflex stimuli.

LONGER-TERM REGULATION BY NERVES OF SALIVARY PROTEIN COMPOSITION

• There is well-documented diurnal variation in the secretory protein content of the rat parotid gland which is associated with the feeding cycle (35,36). 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 (36; Fig. 3). Resynthesis is dependent upon neurally mediated stimuli as it is reduced by feeding rats a liquid diet, which abolishes much of the stimulation

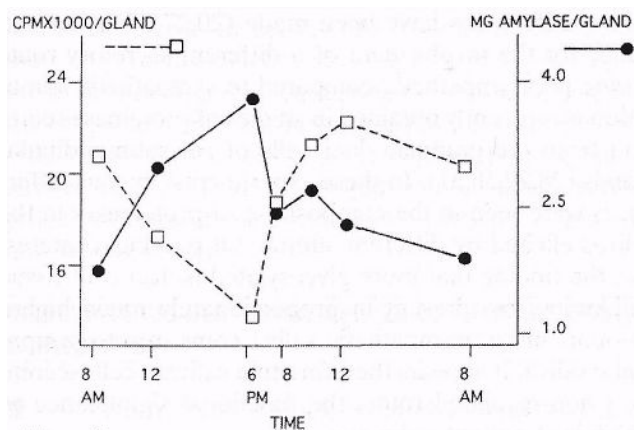


Figure 3.

The diurnal pattern of storage (amylase content) and synthesis (incorporation of label into triachloroacetic acid insoluble material) in the rat parotid gland (modified from Sreebny & Johnson, 1971). The major increases in both protein secretion and resynthesis follow the first feed at the beginning of the dark phase (6pm).

arising from mastication (36). Protein secretion and resynthesis in the rat submandibular gland is not so obviously linked with the feeding cycle, although an increase in the rate of protein synthesis following a feed has recently been demonstrated (37). As with the parotid gland, the reflex secretion of submandibular protein was dependent upon neurally mediated stimuli (38). Protein synthesis in rat parotid and submandibular glands was stimulated by autonomic mimetics (39,40,41,42) and nerve stimulation (26). It remains unclear as to whether protein resynthesis following secretion is regulated at the transcriptional level, translational level or both (43).

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 and synthetic capacity (44,45,46). Such experiments have also demonstrated that the synthesis of different salivary proteins has a varying dependency on neurally mediated stimuli as analysis of the protein components of isoprenaline/pilocarpine-evoked parotid salivas demonstrated compositional changes (45,46,47,48). Thus the proportions of acidic and basic proline-rich protein were reduced as were amylase and ribonuclease, whilst other proteins remained the same (e.g. cysteine-rich protein) or were increased (e.g. deoxyribonuclease and leucine-rich protein). An initial study has indicated that the levels of amylase mRNA were the same or greater relative to total mRNA in the glands of rats fed a liquid diet compared to control-fed rats, suggesting that a transcriptional mechanism was not the cause of reduced levels of enzyme relative to other secretory proteins (49). The protein compositional changes described were shown to be reversed by returning the rats to a normal laboratory chow diet (47). When the opposite manoeuvre was carried out and rats were maintained on a bulk diet, to increase nerve mediated stimuli, no changes in salivary protein composition were seen (50,51). Curiously however, in one report glandular levels of proline-rich proteins were increased in rat parotid glands following maintenance on a bulk diet and this was linked with increases in mRNA levels for these proteins (52). Very few equivalent studies have been performed on humans, however disproportionate reductions in the salivary content of basic proline-rich proteins were reported following 6 weeks of intramaxillary fixation (53).

Feeding a liquid diet results in reductions of stimuli mediated by both parasympathetic and sympathetic nerves. Information concerning the influence of individual branches of the autonomic innervation on salivary protein composition has been obtained through the use of selective denervations, receptor specific pharmacological blockades and agonists. Proctor et al (54) performed uni-

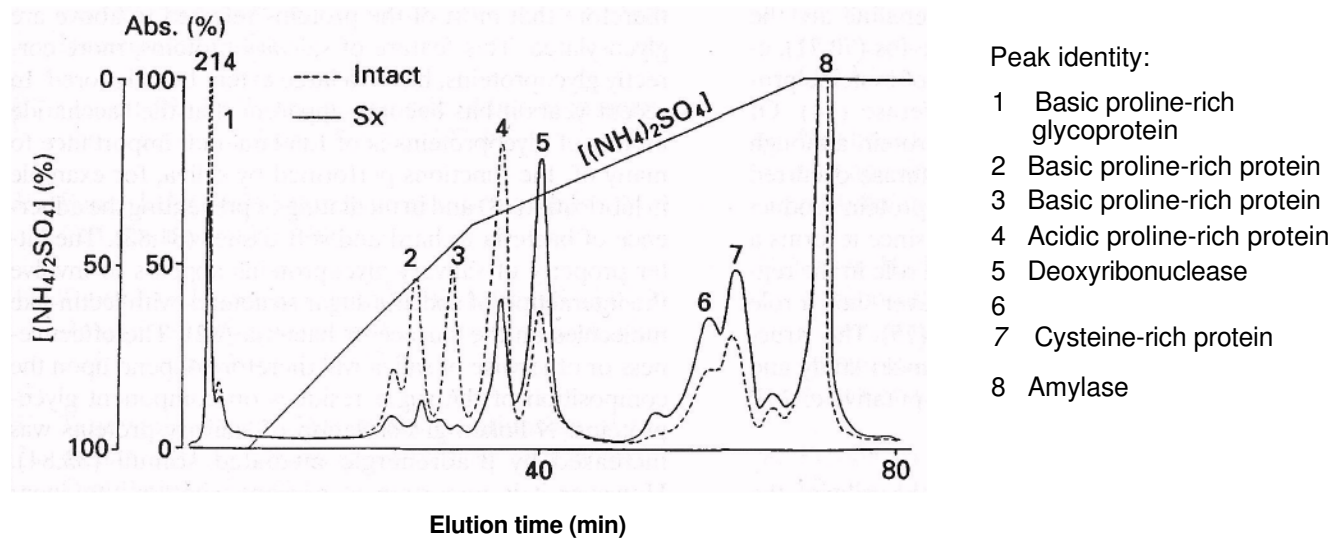


Figure 4.

Comparison of the parotid proteins separated from normal control and chronically pre- or post-ganglionically sympathetomized (Sx) rats, by hydrophobic-interaction chromatography on a phenyl superose column in an FPLC system (taken from Ekstrom et al, 1989). The composition of proteins is dramatically altered following Sx with great decreases in proline-rich proteins and increases in amylase and other resolved proteins.

lateral 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 sympathectomy no significant glandular atrophy took place, nevertheless there was a profound change in the protein composition of saliva. Whilst the amylase specific activity of saliva was increased by almost 50 % the content of acidic proline-rich proteins as a proportion of total protein was reduced to 34 % of the control value. Other proline-rich proteins (basic and basic glycoprotein) were shown to be reduced using SDS gel electrophoresis. Similar changes in composition were observed subsequently in glandular homogenates one week following sympathectomy (55). Following chronic (12 week) sympathectomy the glandular content of proline-rich proteins remained reduced whilst amylase returned to control levels; deoxyribonuclease and cysteine-rich protein were both proportionately elevated (55). These changes were also seen in salivas obtained from chronically sympathectomized rats (56; Fig. 4). 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 chronically (10 days) with the p-adrenoceptor blockers metoprolol or propranolol although under these conditions no elevations in salivary amylase were observed (57).

The great dependence of proline-rich protein synthesis upon sympathetic p-adrenergically mediated stimuli observed in the denervation and antagonist studies above was not entirely surprising. There is now a large body of literature concerning these unusual proteins (3) and the induction of their synthesis in parotid and submandibular glands by pharmacological doses of the p-adrenoceptor agonist isoprenaline (47,58,59,60,61,62). Chronic treatment with isoprenaline has long been known to produce glandular hyperplasia and hypertrophy in rats and mice (63), responses not seen in hamsters despite the similar induction of proline-rich protein synthesis (64). The isoprenaline effect was mediated by the intracellular messenger cAMP and was mimicked by chronic administration of inhibitors of phosphodiesterase, the cAMP degrading enzyme (65). Increased synthesis of existing proline-rich protein and the induction of new proline-rich proteins occurred within one day of isoprenaline injection and was associated with increased mRNA levels (64). A number of proline-rich protein cDNA clones were constructed and the genes from different species have now been sequenced (66,67). The upstream regions of the mouse and hamster proline-rich protein genes contained putative regulatory sequences for cAMP induction (68) and removal of these sequences prevented the isoprenaline-induced proline-rich protein synthesis (69). As such sequences are absent from a characterized human gene (67) it may be that the synthesis of human proline-rich proteins is not dependent on p-mediated stimuli in the same way as the rodent proteins.

Other salivary proteins induced by isoprenaline are the protein products of the proto-oncogenes c-fos (70,71), c-abl and c-sis (71), cystatin S, an inhibitor of cysteine proteinases (72,73) and 4(3-galactosyltransferase (74). Of these only cystatin S is a true secretory protein although the membrane-bound 4p-galactosyltransferase occurred in saliva following its induction (74). The protein product of the proto-oncogene c-fos is of interest since it forms a transcriptional regulator which may play a role in the regulation of the other inducible genes, however such a role in salivary glands has yet to be established (75). The structure of the cystatin S gene is at present under study and this should reveal whether it too contains putative cAMP regulatory elements.

Much less attention has been paid to the role of the parasympathetic innervation and associated neurotransmitters, the most prominent of which is acetylcholine, in the regulation of specific salivary proteins. Parasympathectomy of the cat submandibular gland leads to a disappearance of stored tissue kallikrein in striated ductal cells (6,76) which was later found to be accompanied by massive reductions in the tissue kallikrein content of sympathetically evoked saliva (77). This reduction in the salivary content of tissue kallikrein was seen following chronic muscarinic receptor blockade (78), 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 (79). Amylase specific activity was reduced whilst that of deoxyribonuclease remained unchanged. Chromatography and SDS gel electrophoresis revealed that two basic proline-rich proteins were reduced whilst a basic proline-rich glycoprotein and an acidic proline-rich protein remained unchanged; a further protein (leucine-rich protein) was found to be increased. If the results of this study and earlier studies involving sympathectomy or β -adrenoceptor blockade are considered together, some interesting observations are made. Amylase synthesis appears to show a greater dependence on parasympathetically mediated stimuli whilst the synthesis of two proline-rich proteins in addition to a well-established dependence on p-mediated stimuli requires parasympathetically mediated stimuli. It would be of interest to learn if the synthesis of these proteins was dependent specifically on acetylcholine or on one of the peptide transmitters present in parasympathetic nerves supplying salivary glands (80).

REGULATION OF SECRETORY PROTEIN GLYCOSYLATION

- Salivary secretory proteins are processed through the Golgi complex within secretory cells and it is likely

therefore that most of the proteins referred to above are glycosylated. This feature of salivary proteins, more correctly glycoproteins, has to a large extent been ignored. In recent years it has become apparent that the saccharide moiety of glycoproteins is of fundamental importance to many of the functions performed by saliva, for example in lubrication (8) and in mediating or preventing the adherence of bacteria to hard and soft tissues (81,82). The latter property of salivary glycoproteins appears to involve the interaction of specific sugar structures with lectin-like molecules on the surface of bacteria (82). The effectiveness or otherwise of saliva will therefore depend upon the composition of the sugar residues on component glycoproteins. N-linked glycosylation of salivary proteins was increased by (3-adrenergic mediated stimuli (83,84). However it is uncertain at present whether the sugar sequences of glycoproteins are modified under different physiological conditions by different nerve-mediated stimuli or are invariable and dependent only upon the synthesis of the parent protein.

CONCLUSION

- Salivary protein composition is influenced by neurally mediated stimuli, as demonstrated by many of the studies referred to above. How changes in salivary protein composition effect oral diseases is still unknown, although the protective functions of certain salivary proteins are clear. It is now recognised that protein glycosylation is important in determining the interactions of glycoproteins with bacteria. However the influence of neurally mediated stimuli on such glycosylation has received little attention.

Salivary glands still provide useful models upon which to study the mechanisms of protein secretion in exocrine secretory cells. The influences of exogenous stimuli such as nerve impulses, on resynthesis of individual proteins and the molecular basis of the link between such stimuli and protein synthesis are starting to be elucidated.

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