

Neurogenic factors in the pathogenesis of Sjögren's syndrome

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Neural and endocrine systems may in general participate in the pathogenesis of rheumatic and autoimmune diseases (1). Studies of the hypothalamus-pituitary-adrenocortical (HPA) axis suggest adrenal insufficiency, whereas estrogens function as immunoenhancers and androgens as immunosuppressors. There is evidence for an eventual role of the nervous system and substance P in the so-called neurogenic inflammation (2). This short summary, however, only considers points specific to Sjögren's syndrome (SS).

Salivary gland function is under neural control. Salivary glands are innervated by both postganglionic parasympathetic and postganglionic sympathetic nerves. Both stimulate salivary flow.

The effects of the nervous system on the salivary flow are well known to clinical doctors. One of the most common differential diagnostic alternatives to consider in any patient with dry eyes and dry mouth syndrome is an iatrogenic effect caused by treatment with anti-cholinergic medicines. Usually cardiovascular, psychotropic or anti-allergic drugs are at fault, but it is always expedient to check the patient's medication, because a wide variety of drugs may have anti-cholinergic side effects. Dryness might be caused or aggravated by the patient's medication. In such cases it would seem wrong to treat the patient with systemic SS medication. A natural "cure" is to try to modify the medication so that it does not cause anti-cholinergic dryness.

Sometimes an anti-cholinergic effect would be useful from the patient's point of view. In SS this most commonly occurs when the patient has an interstitial cystitis or an irritable bladder. This together with polydipsia and polyuria may lead to nocturia and disturbed sleep. It has long been suspected that sleep disturbances may, if not cause, at least aggravate musculoskeletal aches and pains and tiredness. In such cases it may sometimes be advisable to use small doses of anticholinergic drugs late in the evening to soothe the urinary bladder, in spite of the side effects.

Another well-known central nervous system effect on salivary flow is caused by psychological distress. Many people suffer from depression, anxiety or burnout in our modern society. This leads to underactivity in the central nervous system salivary centres and to diminished stimulation of the salivary glands and, thus, to dryness. It was earlier thought that these patients would only have a feeling of their eyes and mouth being dry, and that the objective tests, such as the Schirmer-I-test and the measurement of the resting salivary flow, would demonstrate a normal function of their exocrine glands. It now seems that this is not the case. This fact has been one of the reasons why the EU criteria for SS (3), although well validated (4,5), have been criticised. Using the EU criteria, it might be possible to diagnose SS in a patient with subjective complaints of dry eyes and dry mouth, verified by Schirmer's test and sialometry, but with the lack of any autoimmune/ inflammatory findings (such as SS-A/Ro, SS-B/La autoantibodies, RF, ANA, hypergammaglobulinemia, high ESR etc) or symptoms (e.g. atrophic gastritis, autoimmune thyroiditis, interstitial cystitis, renal tubular acidosis type I etc). My personal opinion is that such patients, although they suffer from sicca syndrome and may need symptomatic local treatment, should not be classified as SS. Accordingly, they should not be treated with e.g. oxichloroquine or corticosteroids, but rather with psychotherapy and psychotropic drugs. Theoretically, it is also interesting that neural factors can, based on this clinical observation, be linked to a diminished resting secretion. This implies that also resting, not only stimulated, salivary flow is under nervous control.

A very interesting breakthrough is the discovery of muscarinic acetylcholine receptor modifying autoantibodies, which seem to be present in patients with SS (6,7). In rodents they react with M3-type muscarinic receptors, but studies with muscarinic receptor transfected human cell lines may be necessary until the human equivalent (M1, M2 or M3) can be ascertained. These antibodies may modulate the function of the acinar cells (8,9). If this observation becomes well documented, we may see the sicca facet of the SS in the future as "an autoimmune, antibody-mediated, anticholinergic syndrome".

Apart from the realisation of the role of the nervous system for normal and abnormal salivary gland function, it may be pertinent to know some of the details of this system. Because sialopenia refers to diminished salivary flow, some details of relevance to the regulation of watery salivary flow are depicted in Figure 1.

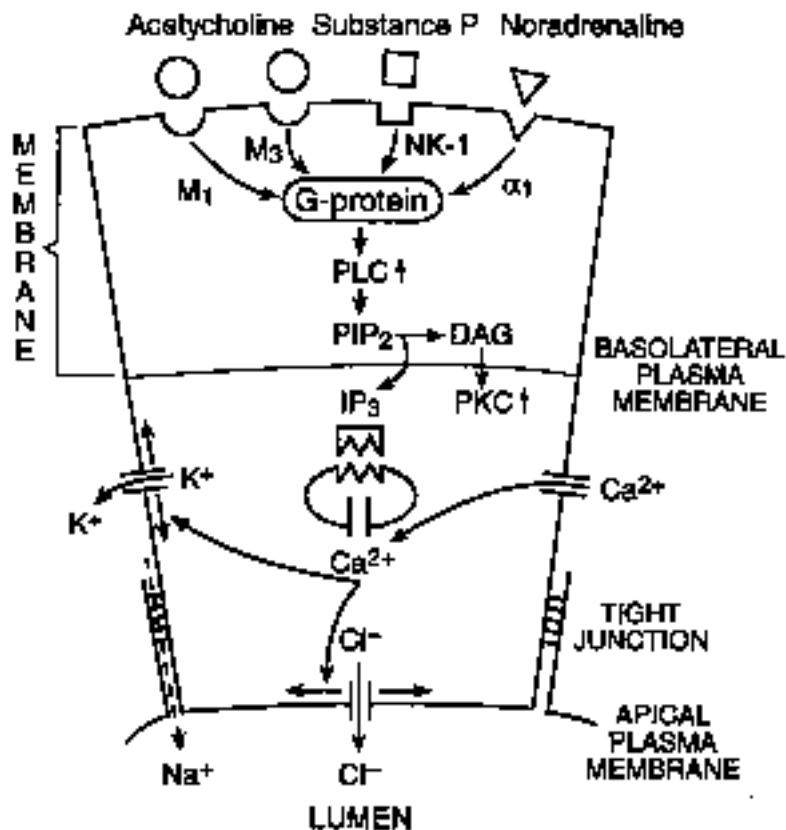


Figure 1. Nervous control of the watery salivary flow from the exocrine acinar cell. For clarity, the basal cell membrane has been drawn out of scale. The most significant stimulatory ligand - receptor interaction is the one between acetylcholine and its muscarinic (of M1-type and in particular of M3-type) receptor. Acetylcholine is released from the postganglionic parasympathetic nerves and, due to the anatomical nerve - acinar cell coupling, is bound to its muscarinic receptor on the cell membrane of its target cell. Other sialogogic ligand - receptor interactions include noradrenaline - (released from the postganglionic sympathetic nerves) α_1 -adrenergic receptor and substance P - NK-1 receptor interactions. All these stimulate the watery salivary flow. They are depicted to be coupled to the same signal transduction system, namely G_{PLC} . The G-protein coupled phospholipase C cleaves plasma membrane-associated phosphatidylinositol 4,5-bisphosphate (PIP_2) to inositol 1,4,5 trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 arm increases the concentration of the cytoplasmic Ca^{2+} and DAG participates in the activation of the protein kinase C system. By an as yet unknown mechanism, the initial, rapid and transient release of Ca^{2+} from intracellular stores is followed by inflow or entry of extracellular Ca^{2+} to the cell. Non-excitable salivary acinar cells seem to lack voltage controlled Ca^{2+} channels. Instead, receptor operated channels (ROC) or, perhaps in particular, second messenger operated channels (SMOC) seem to play a role in the Ca^{2+} entry. Capacitance controlled entrance has been advocated in the presence of high agonist stimulation (Takemura and Putney 1989). Stretch might also play a role in this coupling (Kotera and Brown 1993). Interestingly, in contrast to the biphasic $[Ca^{2+}]_i$ elevations associated with high agonist

concentrations, low and perhaps more physiological agonist concentrations induce "oscillation", i.e. repetitive Ca^{2+} spikes and waves in a response, which may involve arachidonic acid-activated entry (Shuttleworth 1997).

It is of interest that patients with SS lack the α -II isoform of protein kinase C (PKC) in their acinar cells and the α isoform has apparently very low staining intensity (8). There are several possible explanations for this observation, but the most tentative one is to see it as a result of muscarinic receptor stimulation/modulation. It was first thought it might represent efforts to overstimulate the remaining and functionally competent acinar cells, to compensate for the loss of function of disease-affected acinar cells. However, this PKC isoform defect was seen in all patients and was not dependent on the duration or severity of the disease. It was not a focal phenomenon, but was observed in all acinar cells in the diseased glands. The present hypothesis is that it is caused by an autoantibody modulating muscarinic receptors: long-term stimulation of the PKC system leads, after an initial stimulation, to proteolytic degradation of the PKC as a form of negative feedback (10).

Stimulated calcium inflow affects two Ca^{2+} -dependent ion channels, namely the basolateral K^+ channel and the apical Cl^- channel. This, together with constitutively active basolateral $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporter and Na^+/K^+ ATPase, creates an osmotic gradient which "draws" water from the glandular interstitium to the lumen of the acinus (Figure 2). Gross calcium inflow, associated with high agonist concentrations, measured with the use of indicator substances quin 2 and fura 2, has been tentatively shown to be normal in acinar cells from patients with SS.

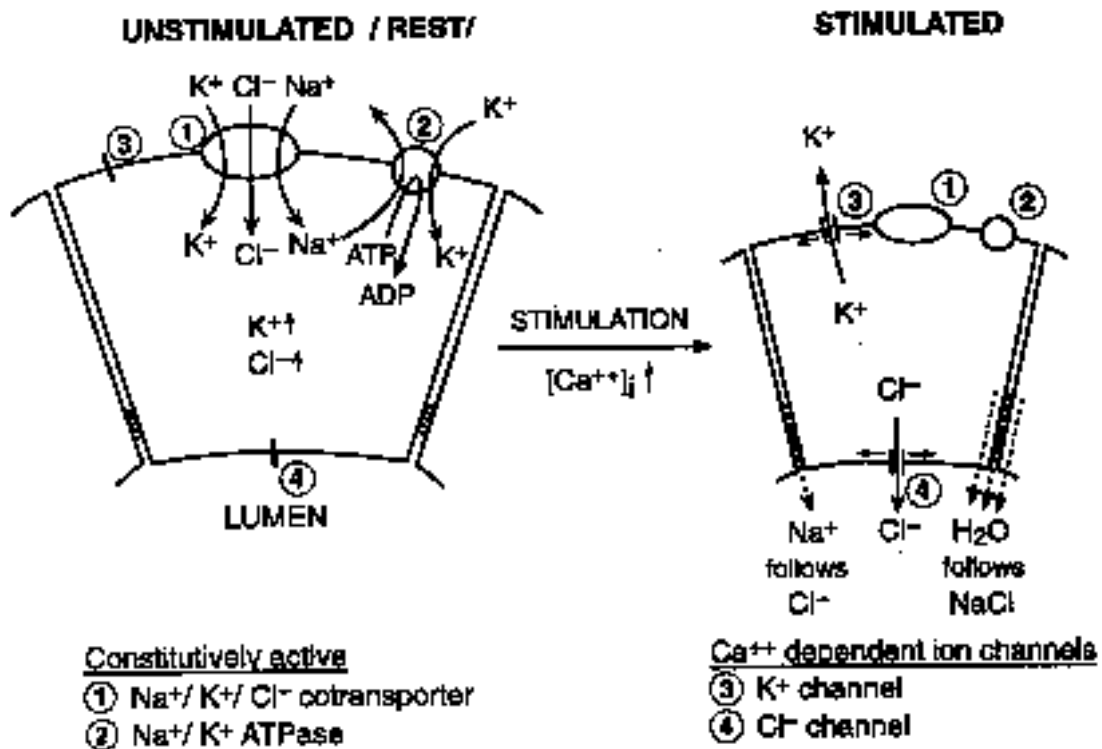


Figure 2. Probably the most important system of ion channels contributing to the formation of the watery primary saliva is often seen centred around a basolateral and constitutively active $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporter (1). This is because among the ion channels this is the best known to clinical doctors. First, it is specifically blocked by loop diuretics, like furosemide and bumetanide. Second, it is used as an uptake route by $^{99\text{m}}\text{Tc}$ -pertechnetate label used in scintigraphies to assess salivary gland function: in case of inflammation/*functio laesa* uptake, concentration, storage and secretion of the label, i.e. the salivary gland function, is seen to be diminished. Na^+/K^+ ATPase (2) contributes to the cellular uptake of K^+ and removes Na^+ at the same time (panel to the left). The Ca^{2+} -gated basolateral K^+ channel (3) and the apical Cl^- (4) channel open up during neural stimulation. Thus, a transacinar osmotic gradient is created.

Interstitial water moves along this osmotic gradient to the lumen of the acinus leading to the formation of the primary saliva (panel to the right).

Because acinar destruction is a pathologic hallmark of the SS, acinar cell apoptosis or programmed cell death might play a part in the loss of a functional glandular tissue or as a source of autoantigen in this condition. Apoptotic acinar cells are readily detected in salivary glands in SS patients. Similarly, a variety of lymphocytes (i.e. CD4⁺ Th1 T cells, CD8⁺, natural killer cells) and other inflammatory cells, which may function as executors for the acinar (resident) cell apoptosis (e.g. via expression and/or release of Fas-L, TNF_α or perforine-granzyme system) are numerous in the focal salivary gland infiltrates in SS. It is noteworthy that acinar cells are structurally isolated from the underlying tissue by the basal lamina, which makes a natural barrier for enacting the cell apoptosis, which requires direct cell-to-cell or ligand-to-cell interaction(s). Other apoptosis pathways, therefore, would seem to be more plausible alternatives in SS. There is evidence that diseased salivary glands in SS are characterised by aberrant co-expression of Fas and Fas-L (11), lack (probably via proteolytic degradation) of PKC (8), a high expression level of inducible nitric oxide synthases (iNOS) and a high local NO production (12), proteolytic derangements in the basal lamina and, as a consequence, loss of a matrix-acinar cell control (13). These factors/conditions (or their net effect) might effectively predispose the acinar cells to apoptosis in SS.

Usually, apoptotic cells are effectively eliminated by macrophages, which utilise $\alpha_v\beta_3$ integrin to capture apoptotic cells without induction of immunoinflammatory stimulation. It still remains to be established how the apoptotic cells are cleared from the tubulo-alveolar system in healthy and affected exocrine tissues. In particular, it will be important to find out whether production of several autoantibodies, local (salivary) interferon- γ production, expression of MHC class II antigen by the ductal epithelial cells (14), and processing of the autoantigens derived from the apoptotic acinar cells are of relevance in SS.

Acinar cells lost via apoptosis and necrosis are usually replaced. It has been suggested that these lost cells are replaced by progenitor cells located in the intercalated ducts. From this site the intercalated duct cells are envisioned to migrate along the basal lamina to the acinar space and to settle down at the site which was previously occupied by the now dead acinar cell. The extracellular matrix (ECM) at this site, which in its composition (e.g. laminin subtype) is probably different from the basal lamina in the intercalated duct, sends via integrin (and maybe also non-integrin) receptors differentiation signals to the cells now at an ectopic site. This ECM-cell interaction stimulates various tyrosine kinases and other signal transduction systems, which alter the phenotype and function of the undifferentiated intercalated duct epithelial cell into that typical for the mature acinar cell. It is, therefore, of interest that not only is the cell substrate, i.e. the basal lamina, affected in SS (13), but also the neurotrophic stimuli in the form of VIP are deficient (15,16). VIP has been speculated to bind to low-affinity, high-capacity receptors, which are coupled to the G_{PLC} system and to trophic effects. VIP mediates the parasympathetic nerve-evoked increase in ornithine decarboxylase activity. This increases the intracellular concentration of growth associated polyamines spermidine and spermine. Many acinar cells in SS have lost this neurotrophic support, due to lack (probably via so called vacuolar degeneration, a phenomenon disclosed in our immunoelectronmicroscopical studies) (17,18) of nerves and peptides in lymphocyte foci. In addition, salivary glands in SS are also otherwise sparsely innervated (*vide infra*).

It has been estimated that normal acinar cells have a half-life of about 200 days and that they are detached to the tubuloalveolar system of the exocrine glands. Due to the direct contact with the outside world (via the excretory duct system), the histological organisation into tubuloalveolar gland and the role of salivary glands for the normal immune function/defence (e.g. production of secretory IgA), they may form a *locus minoris resistentiae* in breakage of autotolerance.

In addition to the system stimulating watery salivary flow, noradrenaline acting on α -adrenergic receptors and vasoactive intestinal peptide acting on high-affinity, low-capacity VIP receptors can stimulate G_s , cAMP formation and thus the PKA system and secretion of protein-rich saliva. It might play a role in the pathogenesis and disease manifestations by altering the composition of the saliva. However, the volumetric effect of this secretion is quite small and it is, therefore, not discussed in more detail.

Another aggravating factor, which via a neurogenic mechanism may contribute to diminished salivary flow in SS is partial loss of the anatomical nerve - acinar cell contact. Innervation of the acinar cells is diminished in the minor salivary glands in patients with SS as revealed by a morphometric analysis of the density of innervation (Figures 3 and 4). Because the minor, rather than the major, glands are responsible for wetting under non-stimulated conditions, this may be a significant pathomechanism in SS. Normal glandular nerves can be seen in epi- and even in hypolemmal localisation. In hypolemmal localisation nude (= not covered by a basal lamina) nerve terminals can be seen located inside the acinar basement membrane, in direct contact with the acinar cell.

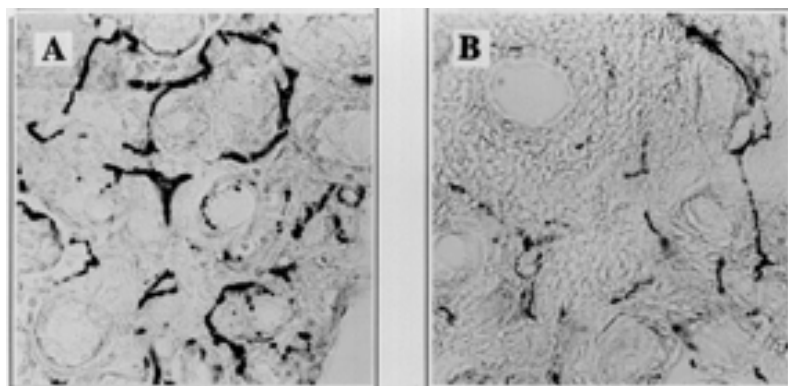


Figure 3. In labial salivary glands vasoactive intestinal peptide can be used as a marker for postganglionic parasympathetic nerves. Panel A demonstrates a normal, healthy labial salivary gland without focal adenitis. Panel B demonstrates a gland from a patient with Sjögren's syndrome. Note the periductal lymphocyte infiltrate and the greatly diminished density of innervation. Figure 4 gives some quantitative results of these types of samples.

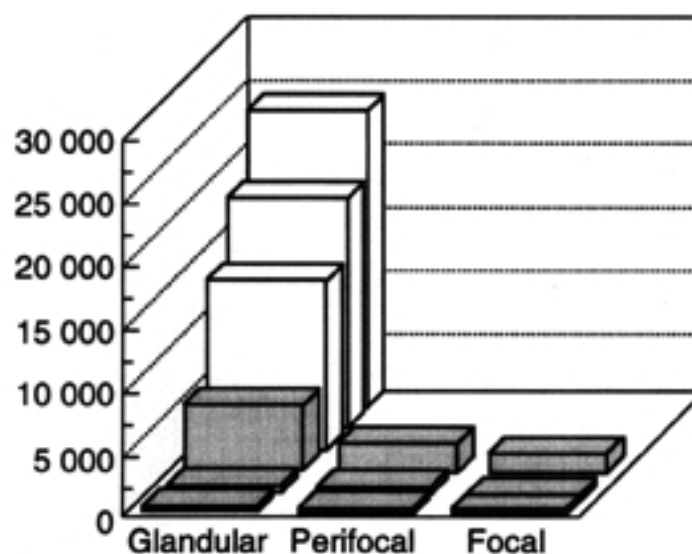


Figure 4. The figure gives the intersection point count (a measure of the length of the nerves in the sample) in three normal and three diseased SS: notice the clear difference in favour of the normal glands. Labial salivary glands are sparsely innervated in patients with SS.

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