Neuropeptides of the autonomic nervous system in Sjögren’s syndrome

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Abstract
Objective—To assess the activity level of the autonomic nervous system in Sjögren’s syndrome (SS) and to correlate this with stress.

Methods—Patients with SS (n=12) and healthy controls (n=10) were analysed for the content of vasoactive intestinal peptide (VIP) and neuropeptide Y (NPY) in their stimulated saliva by radioimmunoassays and for stress by the use of a modified Jenkins Activity Survey (JAS).

Results—The data are expressed as median (interquartile range). Salivary VIP output (pg/min) and NPY output (pg/min) were high in SS compared with healthy controls (30.0 (15.6, 36.6) versus 12.3 (9.2, 24.0), p=0.045, 4.8 (0.6, 24.1) versus 0.7 (0.0, 2.4), p=0.038, respectively). Patients experienced only a little, but not significantly, more stress than the healthy controls (stress index −2.8 (−7.7, 6.9) versus −5.2 (−12.9, 2.7), p>0.05). Stress in general was associated with high salivary VIP concentrations (r=0.41, p=0.05).

Conclusions—These findings show that adequately processed saliva (containing aprotinin and EDTA as neuropeptidase inhibitors) contains measurable amounts of marker peptides of the autonomic nervous system. Secondly, VIP concentration but not output may be affected by stress, which may act by decreasing salivary flow. In patients with SS, VIP and NPY outputs are increased. This may indicate increased leakage into saliva or efforts to compensate for the diminished salivary flow, or both.

Sjögren’s syndrome (SS) is a systemic autoimmune disease characterised by exocrinopathy of unknown aetiology and pathogenesis leading to dry mouth (xerostomia) and dry eyes (keratoconjunctivitis sicca). A particularly prominent and diagnostically useful feature is focal adenitis, which develops in both salivary and lacrimal glands. For practical reasons focal adenitis is usually diagnosed from labial salivary gland biopsy specimens. For practical reasons focal adenitis is usually diagnosed from labial salivary gland biopsy specimens. For practical reasons focal adenitis is usually diagnosed from labial salivary gland biopsy specimens. For practical reasons focal adenitis is usually diagnosed from labial salivary gland biopsy specimens. For practical reasons focal adenitis is usually diagnosed from labial salivary gland biopsy specimens.

There is a remarkable discrepancy between the focal and often relatively mild glandular involvement in form of adenitis (with pathological focus score values ≥1 often affecting less than 10% of the total area, except in far advanced end stage cases) and the considerably decreased salivary flow (≤0.1 ml/ml of whole resting salivary flow—that is, about only 10% of the normal salivary production). One explanation, which has recently been put forward, is that this autoimmune disorder as such or as a result of focal adenitis leads to vasoneural dysregulation and injury of the peripheral nerve fibres and thus to diminished salivary flow and atrophy of the acinar cells. It has been widely accepted that normal salivary flow is under neural regulation: parasympathetic stimulation increases salivary flow, whereas stressful stimuli decrease it via central sympathetic activation.

Accordingly, a parasympathomimetic drug, pilocarpine, has been found useful in the treatment of dry mouth and electrostimulation, which augments normal physiological salivary reflexes, stimulates production of saliva in SS. The aim of the study was therefore to measure the salivary content of vasoactive intestinal peptide (VIP) and neuropeptide Y (NPY), which in labial salivary glands are found mainly in the parasympathetic and sympathetic arm, respectively, of the autonomic nervous system. Activity of the autonomic nervous system is influenced by the somatic well being, in particular by the degree of stress. In fact, dry mouth associated with depression and anxiety or the treatment with psychotropic drugs (with anticholinergic side effects), or both, is one of the most important differential diagnostic alternatives in Sjögren’s syndrome clinics. We therefore also aimed to measure stress by using a modified Jenkins Activity Survey (JAS) to correlate it with the degree of sympathetic and parasympathetic activity.

Methods
PATIENTS AND CONTROLS
Twelve female patients with chronic established SS were studied; all fulfilled the European Classification Criteria for SS. Primary SS was diagnosed if any four of the six classification criteria are fulfilled. Secondary SS is diagnosed if there is a positive response to items 1 or 2 (ocular symptoms or oral symptoms), plus a positive response to at least two items from among 3, 4, and 5 (ocular signs, histopathological features, salivary gland involvement). In both cases, the adequate exclusions (sarcoidosis, lymphoma, acquired immunodeficiency syndrome and graft versus host disease) were done. Ten healthy female volunteers served as controls.
The average age of the SS patients was 47.5 (range 21–59) years. The duration of the SS was on average 8.6 (range 1–17) years. All the patients were non-smokers. Five of the patients were married, three unmarried, and four divorced. Three of them worked in a white collar profession, four in a blue collar profession, one was a college student, and four were retired because of the disease.

The average age of the 10 healthy controls was 47.7 (range 39–56) years. Six of them were married, one was engaged to be married, two were divorced, and one was a widow. Nine of the controls were blue collar workers in a health care profession and one was a cleaning woman. Eight of them were non-smokers, one smoked 5–20 cigarettes a day, and another smoked more than 20 cigarettes.

**Results**

**SALIVARY FLOW RATE**

There was no significant difference in the median between the stimulated flow rate (ml/min) of the SS patients and the healthy controls (0.4 (0.2–0.7) v 0.4 (0.3–0.4), p=0.304), but there was a significant difference in the variability between the flow rate values of the groups (p = 0.0018) (fig 1).

**SALIVARY VIP AND NPY**

Salivary VIP output (pg/min) was high in SS compared with healthy controls (30.0 (15.6–36.6) v 12.3 (9.2–24.0), p=0.045) with no difference in variability (p=0.11) (fig 2). Salivary VIP concentration (pg/ml) was higher in SS compared with healthy controls (4.8 (0.6–24.1) v 0.7 (0.0–2.4), p=0.038), also there was significant difference in variability (ip=0.016) (fig 3). Salivary NPY concentration (3.4 (1.2–76.0) pg/ml v 2.1 (0.0–7.1) pg/ml)
and content (pg/mg protein: 2.3 (0.6–27.3) v 2.7 (1.6–4.4)) did not significantly differ in SS and in healthy controls and there were no differences in variability. NPY contents in the two smokers were similar to NPY values in other healthy controls.

**Discussion**

SS is characterised by sialopenia/xerostomia. It is the resting salivary flow, however, which is low. Stimulated salivary flow varies a lot between patients and may be normal. It has therefore not been included in the European Classification criteria. As a matter of fact, many patients use sialogogic stimuli, mechanical (such as a xylitol chewing gum) or chemical (such as pilocarpin) to alleviate their symptoms. In advanced or difficult cases, or both, stimulated salivary flow/reserves are low, but in many series including newly diagnosed patients stimulated salivary flow is normal. This was also the case in our series.

VIP is a 28 amino acid peptide, which belongs to the family of brain-gut peptides. It in salivary glands it often colocalises with choline acetyltransferase and seems therefore to be useful as a peptide marker for postganglionic parasympathetic fibres in that tissue. NPY is a 36 amino acid peptide of the NPY/CPON family. It usually colocalises with cytoplasmic tyrosine hydroxylase and intravesicular dopamine-β-hydroxylase, which are classic markers for postganglionic sympathetic nerves. Both VIP and NPY containing peptidergic nerves have been recently described in salivary glands in normal healthy controls and in patients with SS.

In this study the eventual release into and presence in saliva of VIP and NPY was assessed by RIA from saliva collected to a cocktail of enzyme inhibitors selected to prevent artefactual in vitro degradation by various neuropeptidases. Both neuropeptides were found in the whole series, stress score (as expressed by the JAS factor A-B type behavior) correlated significantly with both VIP concentration (pg/ml, r=0.41, p=0.05) and content (pg/mg protein, r=0.53, p=0.02). Thus, the more stress, the higher the VIP values. The correlation between NPY concentration or content and stress did not quite reach significance (r=0.25, p>0.05; r=0.33, p>0.05).

When all study subjects were subdivided into patients and controls, it was found that in the patient group there were no significant correlations between the stress score and VIP values in the saliva, although there was a significant correlation between the stress score and the NPY concentration (r=0.66, p=0.02). There were positive, but not statistically significant, correlations in the healthy controls between the stress score and the VIP content and the VIP output (r=0.52, p>0.05; r=0.54, p>0.05 respectively).

**Correlation between salivary neuropeptides and stress**

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adequately processed samples suggesting local release and secretion to saliva.

After this first finding on the presence of VIP and NPY in saliva, eventual effects of disease and stress on their concentration were evaluated. These are often connected, because psychosocial features connected with chronic diseases contribute to psychic and somatic well being. The JAS instrument describes the person's stress behaviour. The type A behavioural pattern comprises a cluster of actions and emotions exhibited by people who engage themselves in a chronic struggle and have a hyperresponsiveness to almost any challenge. These people are competitive with a driving need for control and an accentuated sense of time urgency. Changes in lifestyle or circumstances as well as disease are known to modify the person's type A behavioural pattern. While stress may affect health, there is also evidence that a state of ill health, as was the case with our SS patients, can act as a significant source of stress in itself or can sensitize the person to other sources of stress by reducing their ability to cope. Furthermore, at any point of time, the relation between stress and disease can operate in both directions, and may develop in a vicious cycle.

SS patients experienced on average slightly but not significantly more stress than the healthy controls as measured with the JAS instrument. According to the present findings, patients with SS seem to cope surprisingly well with their disease, at least as assessed by the extent of the stress they experience.

The physiological factors regulating VIP and NPY secretion are not known. In this study we found an interesting correlation between stress and salivary VIP concentration and content, but not VIP output. Stress might increase VIP release or, more probably, decrease the watery salivary flow. Stress may interfere with the normal salivary gland function finely controlled by coordinated and balanced excitatory and inhibitory inputs from tonically active postganglionic neurons.

VIP has many different paracrine effects. It induces vasodilatation, modulates mast cell mediator release and lymphocyte migration and recirculation. In addition, VIP has important trophic effects on salivary gland acinar cells in the long term. According to recent immunoelectron microscopic studies published elsewhere, VIPergic fibres come into close contact with the acinar cells and some fibres are even located hypolemmally—that is, inside the acinar basement membrane. VP causes vasoconstriction, modulates neurotransmitter release from sensory and postganglionic sympathetic neurons, and up regulates adhesiveness of endothelial cells for leucocytes. According to this study, these neuropeptides are not only found in salivary glands, but are also released there and to some extent found in saliva; they might therefore contribute to the local pathomechanisms in SS.