Sensory stimulation (acupuncture) increases the release of calcitonin gene-related peptide in the saliva of xerostomia sufferers

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Summary Over the last decade, several patients afflicted with xerostomia have been treated with acupuncture. Their salivary flow rates increased significantly and the improvement lasted during a long observation period. We also found that the release of several neuropeptides in the saliva of healthy subjects can be increased by acupuncture stimulation. The concentration of vasoactive intestinal polypeptide increased significantly in the saliva of xerostomic patients after acupuncture treatment. The release of the neuropeptide calcitonin gene-related peptide (CGRP) was investigated in the saliva of xerostomic patients in order to elucidate further the mechanisms of the effect of sensory stimulation (acupuncture) on the salivary secretion. CGRP-like immunoreactivity was measured with radioimmunoassay (RIA) before and after a double series of acupuncture treatment, in stimulated saliva of 14 patients who suffered from xerostomia. The results showed that the concentration of CGRP increased significantly (P<0.001) in the saliva of these patients after the end of acupuncture treatment as compared to base-line levels. Taking into consideration the influence of CGRP on the salivary flow, as well as its trophic effect, we concluded that the increased release of CGRP could be one of the factors that affect positively the salivary flow rates of xerostomic patients who were treated with acupuncture.

INTRODUCTION

Severe xerostomia (dry mouth) is a well known symptom of some systemic diseases, e.g. primary and secondary Sjögren's Syndrome, as well as an unwanted side-effect of radiation treatment or medication. It causes many problems for the affected patient—difficulties in speaking, eating and swallowing, rampant caries and infections of the oral mucosa, inability to wear dentures, altered sense of taste, and can be both physically and socially handicapping. Different kinds of techniques have been developed in order to deal with xerostomia; stimulation of salivary secretion by chewing gums or tablets, and saliva substitutes ranging between plain water and ex tempore concoctions that try to mimic natural saliva. Medications, such as pilocarpine and nicotinamide are sometimes used. Battery operated devices applied to the palate and tongue have also been tried. All these methods have one thing in common: their alleviating effect lasts only for a short period of time. Over the last decade we have treated patients with xerostomia of different aetiologies with acupuncture. The results showed that their salivary flow rates can be increased with this method and that the improvement often lasts for long periods; a year or longer.

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different parts of the nervous system; both the sensory and motor system of the central nervous system (CNS), as well as the autonomic nervous system (ANS).18–25

Several studies have been conducted on the effect of acupuncture in the treatment of ischemic conditions, and it has been shown that acupuncture stimulation exerts its effect by increasing the release of neuropeptides from nerve endings.26–31 It has also been shown that acupuncture can mimic muscle exercise.32,33

One of the neuropeptides that were affected by sensory stimulation was calcitonin gene-related peptide (CGRP). This peptide consists of 37 amino acids, and it wields a wide range of effects on gastrointestinal, endocrine, cardiovascular and broncho-tracheal systems, as well as on CNS and ANS. CGRP is involved in neurogenic inflammatory reactions and modulates response to substance P (SP) in neurogenic inflammation; it causes endogenous vasodilatation and acts by releasing an endothelium derived relaxing factor (EDRF), but not cyclic GMP. CGRP does not cause extravasation but strongly potentiates the increase in extravasation produced by SP and potentiates the oedema induced by SP. It also has a relaxant effect on vascular smooth muscle and can increase the blood flow in the skin for several hours. CGRP is a potent vasodilator, induces protracted increase in microvascular blood flow, and potentiates the increase in capillary permeability produced by platelet activation factors. CGRP co-exists with SP in many primary afferents and monitors noxious events together with SP. It plays a part in pain perception; potentiates the hyperalgesia caused by SP and conveys nociceptive information to the first synapse in the spinal cord, where pain is processed.26,34–36 CGRP induces a delayed (1–2 min) increase in salivary secretion,35 and its concentration in the saliva can be affected by pathology.37

The salivary secretion is controlled by the ANS where the transmitter substances are acetylcholine (Ach) and noradrenaline (NA). The parasympathetic system is dominant and it induces a powerful increase of the salivary secretion rates with low protein content. It causes contraction of myoepitelial cells and vasodilatation of capillaries in the salivary glands, which leads to increased blood flow. The sympathetic system has a more intermittent role; when activated it causes a low, viscous salivary flow.38–43

In order to elucidate the mechanisms behind the effect of acupuncture on salivary secretion in xerostomia sufferers, we conducted several studies concerning the connection between salivary secretion, neuropeptides and acupuncture. We found that acupuncture stimulation increases the blood flux in the skin over parotid gland in patients with Sjögren’s syndrome.50

The neuropeptide concentration in saliva varies with secretion rates51 and we have shown that the concentration of some neuropeptides can be increased in the saliva of healthy subjects with acupuncture stimulation.52

In a recent study, we found that the concentration of vasoactive intestinal polypeptide (VIP) increased after the acupuncture treatment as compared with base line levels, in the saliva of xerostomic patients.53 In this study, we investigated the influence of acupuncture stimulation on the concentration of CGRP in the saliva of xerostomia sufferers. We hypothesized that acupuncture stimulation could increase the release of CGRP from the nerve endings of the autonomic nervous system by the way of

<table>
<thead>
<tr>
<th>Patient/Gender</th>
<th>Age</th>
<th>Time of xerostomia</th>
<th>Aetiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. female</td>
<td>57</td>
<td>2 years</td>
<td>Primary Sjögren's Syndrome</td>
</tr>
<tr>
<td>2. male</td>
<td>71</td>
<td>10 years</td>
<td>Primary Sjögren's Syndrome</td>
</tr>
<tr>
<td>3. female</td>
<td>38</td>
<td>10 years</td>
<td>Unknown</td>
</tr>
<tr>
<td>4. female</td>
<td>63</td>
<td>10 years</td>
<td>Primary Sjögren's Syndrome</td>
</tr>
<tr>
<td>5. male</td>
<td>55</td>
<td>1.5 years</td>
<td>SLE</td>
</tr>
<tr>
<td>6. female</td>
<td>54</td>
<td>8 years</td>
<td>Primary Sjögren's Syndrome</td>
</tr>
<tr>
<td>7. male</td>
<td>45</td>
<td>4 months</td>
<td>Radiation treatment</td>
</tr>
<tr>
<td>8. female</td>
<td>54</td>
<td>9 months</td>
<td>Radiation treatment</td>
</tr>
<tr>
<td>9. female</td>
<td>57</td>
<td>13 months</td>
<td>Radiation treatment</td>
</tr>
<tr>
<td>10. male</td>
<td>37</td>
<td>17 months</td>
<td>Radiation treatment</td>
</tr>
<tr>
<td>11. female</td>
<td>63</td>
<td>8 months</td>
<td>Radiation treatment</td>
</tr>
<tr>
<td>12. male</td>
<td>54</td>
<td>13 months</td>
<td>Radiation treatment</td>
</tr>
<tr>
<td>13. male</td>
<td>65</td>
<td>2.5 years</td>
<td>Radiation treatment</td>
</tr>
<tr>
<td>14. female</td>
<td>66</td>
<td>2 years</td>
<td>Radiation treatment</td>
</tr>
</tbody>
</table>
excitation of sensory efferent nerve fibres, thus leading to an increase of salivary flow.

MATERIALS AND METHODS

Patients

Out of 65 patients that were treated with acupuncture for xerostomia, 17 were chosen to participate in this study due to their ability to produce enough stimulated saliva for the radioimmunoassay (RIA) analyses prior to the start of the study. We were able to conduct RIA analyses of CGRP on the saliva of 14 of these patients, six men and eight women, aged 37–71, median age 56 years. The periods of time that they have suffered from xerostomia differed from a few months (after radiation treatment) to several years for the SS patients. Patient data are shown in Table 1. The experimental design was approved by the ethics committee at the Huddinge Hospital.

Saliva collection

Using the standardized salivary flow test procedures used at the department of Cariology,54 we collected paraffin-chewing stimulated saliva from the patients before the beginning of the study and then after the acupuncture treatment was completed. The patients were informed about the study in writing and were asked to refrain from eating, drinking and smoking for at least 1 h prior to each experiment. Each individual participating in the study was tested at approximately the same time of the day considering the differences in saliva production during the day. The collected saliva was weighed in order to obtain precise measurements (1 g was considered to respond to 1 ml). The saliva samples were collected in small test tubes, containing 1 ml 1 M acetic acid in order to neutralize the enzymes that would otherwise destroy the neuropeptides. The samples were kept in ice during the experiment and were frozen to −70°C immediately after the end of each session, until the radioimmunoassay analyses (RIA) could be carried out at a later date.

Acupuncture treatment

The acupuncture treatment was performed by an experienced acupuncturist, and the same points were used as in earlier studies when xerostomia was treated with sensory stimulation.15–17 The following points were used bilaterally St3, St6 in the face, Li4 in the hands and St36 and Sp6 in the legs. They are described in detail Table 2.55 Disposable, Chinese needles were used, made of stainless steel, Hwato 0.32 × 40 mm, and Cloud & Dragon, 0.30 × 15, 30 and 40 mm. After standard disinfecting of the site, the needles were inserted through the skin to the depth of 5–10 mm, and manipulated until the needle sensation (DeQi) was reached. DeQi is described as a feeling of heaviness and ache, sometimes combined with the sensation of a current originating from the point where the tip of the needle is placed.56 When that sensation was achieved, the needles were left in situ, and not manipulated again unless a needle was displaced. All patients received two series of acupuncture treatments – 24 sessions of 20 min each.

Peptide analyses

Samples from the patients’ saliva were extracted using a reverse-phase C18 cartridge (Sep Pak, Waters) and

Table 2 The most commonly used acupuncture points

<table>
<thead>
<tr>
<th>AP-Point</th>
<th>Location</th>
<th>Tissues</th>
<th>Skin Innervation</th>
<th>Muscle Innervation</th>
</tr>
</thead>
<tbody>
<tr>
<td>St 3 Juliao</td>
<td>When the eyes look straight forwards, it is inferior to the pupilla at the level of the lower border of the alae nasi</td>
<td>m. levator labii</td>
<td>n. trigeminus, r. ophthalminus, n. infraorbitalis</td>
<td>n. facialis</td>
</tr>
<tr>
<td>St 6 Jiache</td>
<td>One finger width anterior and superior to the angle of lower jaw, at the prominence of the m. masseter during mastication</td>
<td>m. masseter</td>
<td>n. trigeminus, r. mandibularis</td>
<td>n. facialis, r. mandibularis, n. trigeminus</td>
</tr>
<tr>
<td>Li 4 Hugu</td>
<td>On the middle point of os metacarpale II, on the prominence of the 1st m. interossei dorsales slightly towards the side of the index.</td>
<td>m. intersosseus dors., m. abduct. pollicis, m. lumb.ocr.</td>
<td>n. radialis, r. superficialis</td>
<td>C 6, 7, 8</td>
</tr>
<tr>
<td>St 36 Zusani</td>
<td>10 cm below apex patellae, one finger width lateral to the crista anterior tibiae</td>
<td>m. tibialis ant.</td>
<td>n. cutaneus lat.</td>
<td>L 5, S 1,2</td>
</tr>
<tr>
<td>Sp 6 Sanyinjiao</td>
<td>10 cm above the highest point of the malleolus medialis at the posterior border of the tibia</td>
<td>m. flexor digit. long.</td>
<td>n. sapheneus</td>
<td>L 3,4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>m. tibialis post.</td>
<td>n. tibialis</td>
<td>L 4, S 2, 3</td>
</tr>
</tbody>
</table>
analysed using competitive radioimmunoassay (RIA). Calcitonin gene-related peptide (CGRP-LI) was analysed using antiserum CGPR8 raised against conjugated rat CGRP. HPLC-purified \textsuperscript{125}I-Histidyl rat CGRP was used as radioligand, and rat CGRP as standard. The crossreactivity of the assay to SP, neurokinin A, neurokinin B, neuropeptide K, gastrin, neurotensin, bombesin, neuropeptide Y and calcitonin was less than 0.01%. Crossreactivity towards human CGRP alpha and beta was 93 and 24%, respectively, and toward rat CGRP alpha and beta 100 and 120%, respectively. Intra- and interassay coefficients of variation were 8 and 14%, respectively.\textsuperscript{57,58} The lower detection limit in all extracted samples was 0.1 fmol/mL for all peptide assessments.\textsuperscript{59}

**Statistical analyses**

Wilcoxon's test (paired two samples) was used in order to compare the concentrations of CGRP in the saliva before the beginning of the study and after the acupuncture treatment was completed. A probability value of \( P<0.05 \) was considered significant.

**RESULTS**

The concentrations of CGRP in the saliva of the patients in this study were significantly higher after the end of acupuncture treatment as compared to the base line levels, before the AP was started (\( P<0.001 \)). The results are visualized in Figure 1. HPLC showed a distinct immunoreactive component with regard to CGRP, as reflected by a main peak eluting in the position of the corresponding synthetic peptide. No evidence was found to indicate presence of multiple immunoreactive components (Fig. 2).

**DISCUSSION**

In earlier studies, we found that acupuncture treatment can increase the salivary secretion in patients who suffer from xerostomia. The results of those studies showed that the improved salivary flow rates persist during a long period – a year or longer.\textsuperscript{15–17} In this study, we investigated the influence of sensory stimulation on the release of CGRP in the saliva of xerostomic patients. We found that the concentration of CGRP was significantly increased in the stimulated saliva of those patients after the end of acupuncture treatment as compared to the levels before the start of the study. The salivary secretion is controlled by the ANS, in complicated co-operation with the sensory afferents and CNS. The afferent impulses from the masticatory system activate the...
parasympathetic and sympathetic systems of ANS, thus inducing an increase in the salivary flow. The salivary neurons in the lateral reticular formation of the lower brain stem are activated by the stimulation of group II and group II afferent nerve fibres of n. trigeminus.

CGRP, one of the neurotransmitters found in the nerve fibres of both the sensory and autonomic nervous system, beside Ach and NA, is involved in salivary secretion. We have shown in earlier studies that the concentration of CGRP in the saliva of healthy human subjects vary with the stimulation of salivary flow rates, and can be increased significantly by acupuncture stimulation.

We found in this study that the concentration of CGRP can also be increased significantly by acupuncture stimulation in the saliva of xerostomic patients. We speculated therefore that the mechanism behind this effect might be the excitation of the afferent fibres by sensory stimulation. Upon stimulation the afferents release significant amounts of CGRP locally. There is also a release of CGRP from the motor efferents of the masticatory system, which are activated by the afferent input.

This increased release of the neuropeptide CGRP could be one of the factors that contribute to the salivary secretion. We have suggested in the preceding study that VIP can act as a trophic factor on the salivary gland tissues. It is possible that CGRP has a similar function apart from its influence on the salivary secretion rates. According to Dalsgaard et al., CGRP has trophic influence on the endothelial cells, and might positively affect the microcirculation in the capillary vessels. The salivary glands contain considerable amounts of neurotrophins, which exert profound trophic effect on the neurons of the ANS and the sensory nervous system.

Acupuncture stimulation may produce a release of the neurotrophins which affect the sensory and autonomic NS by the way of inducing an increase in the production of CGRP in the nerve cells and subsequent release of that CGRP in target organs, in this case the salivary glands. Such course might be the explanation of the long-term effect of acupuncture treatment.

We concluded that the increase of the release of CGRP could be one of the factors that contribute to the improvement of the salivary secretion rates in xerostomic patients who were treated with acupuncture. The mechanisms behind the effect of the sensory stimulation might be the activation of the nerve fibres of the systems involved, namely sensory afferents as well as the motor and efferent neurons, leading to an increased release of the neuropeptide CGRP, which could have a dual effect. A direct influence on the salivary flow rates, as well as a long-term influence on the condition of the salivary gland tissues through its trophic influence on the endothelial cells of the blood vessels, resulting in improvement of the blood supply of the salivary glands.

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REFERENCES

24. Kaada B. Neuropsychological mechanisms of pain suppression and cutaneous vasodilation induced by transcutaneous nerve stimulation (TNS) and acupuncture – a review. Legeivitskap og livsvisdom 1982; 64–94.


