Sensory stimulation (acupuncture) increases the release of vasoactive intestinal polypeptide in the saliva of xerostomia sufferers

I. Dawidson, B. Angmar-Månsson, M. Blom, E. Theodorsson, T. Lundeberg

1Department of Cariology, Karolinska Institutet, Box 4064, 141 04 Huddinge, Sweden
2Clinical Chemistry, University Hospital, Linköping, Sweden
3Departments of Physiology and Pharmacology and Surgery and Rehabilitation, Karolinska Institutet, 104 01 Stockholm, Sweden

Summary We have shown in earlier studies that xerostomia can be treated successfully with acupuncture. We also found that acupuncture stimulation can increase the concentration of neuropeptides in the saliva of healthy subjects. In this study, the concentration of the neuropeptide vasoactive intestinal polypeptide (VIP) was measured in the saliva of xerostomic patients in connection with acupuncture treatment (AP). Patients suffering from xerostomia caused by irradiation treatment, Sjögren's syndrome and other systemic disorders had been treated with acupuncture. Some of these patients showed an increase of their salivary flow rates after the AP was completed. Seventeen patients out of 65 were chosen due to their ability to produce enough saliva for the radio immunoassay (RIA) analyses to be conducted prior to the start of AP. VIP-like immunoreactivity (VIP-LI) was measured in the chewing stimulated saliva of these patients before and after the whole AP (24 sessions of 30 min each). The results showed that there was a significant increase of the concentration of VIP after the AP as compared to the measurements made before the start of the treatment (p<0.05).

We concluded that the increase of neuropeptide VIP might be one of the mechanisms behind the positive effect of acupuncture on the salivary flow rates of the xerostomic patients.

INTRODUCTION

An insufficient production of saliva is an extremely unpleasant symptom of several systemic diseases, of which primary and secondary Sjögren's syndrome are the most common. The salivary glands of xerostomia sufferers become severely impaired by chronic inflammatory process.1-3 Patients who have been treated with radiation therapy for head and neck tumours are similarly afflicted.4 Several patients suffering from xerostomia have been treated with acupuncture over the last decade. The results of those studies showed that the salivary flow rates of many of these patients increased after the treatment and the positive results persevered during a long observation period.5-7 The functions of the salivary glands are controlled by both the sensory and autonomic nervous systems. The parasympathetic system has the main influence on the salivary secretion.8-12 Several studies have shown that the salivary flow rates can be affected by neuropeptides.13-18

It has also been shown that neuropeptides substance P (SP) and vasoactive intestinal polypeptide (VIP) prevented the fall in the parotid gland weight after parasympathetic denervation and that VIP prevented the fall in the sublingual gland weight induced by liquid diet. These results suggest a trophic role for SP and VIP on parotid gland and for VIP on sublingual gland, which can also be exerted naturally as a result of their release from nerve fibres containing these neuropeptides around the acini.19 The release of these neuropeptides in human saliva has been investigated and the results of that study showed that the concentration of the neuropeptides in the saliva of healthy subjects varies with the mode of stimulation.20 Furthermore, it was found that the release of neuropeptides in the saliva of those healthy subjects

Received 3 July 1998
Accepted 13 August 1998

Correspondence to: Irena Dawidson, Department of Cariology, Department of Odontology, Karolinska Institutet, Box 4064, S-141 04 Huddinge, Sweden. Tel: +46 8 728 8179, Fax: +46 746 7081, e-mail: Irena.Dawidson@ofa.ki.se
could be increased by acupuncture stimulation.\(^{21}\) VIP, one of the investigated neuropeptides found in the parasympathetic nervous system, plays a crucial role. Vasoactive intestinal polypeptide has been so called due to its vasoactive properties.\(^{22,23}\) It has anti-inflammatory and immunoregulatory properties,\(^{24,25}\) and influences salivary flow.\(^{26-34}\) The concentration of VIP in the saliva of xerostomic patients who were treated with acupuncture was investigated in this study. We hypothesized that sensory stimulation through reflexes activates parasympathetic efferents, increasing the release of VIP which apart from stimulating salivary secretion, inhibits the inflammation and has also a trophic influence on the gland tissue, thus leading to an increase of the salivary flow rates in the xerostomic patients.

**MATERIALS AND METHODS**

Prior to the beginning of the study, the ethical committee at Huddinge Hospital, approved the experimental design. The participants were informed about the study in writing and that they could withdraw at any moment.

**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 radio immunoassay (RIA) analyses prior to the start of the study. We were able to conduct RIA analyses of VIP on the saliva of 10 of these patients, four men and six women. Patient data are shown in Table 1.

<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>PSS</td>
</tr>
<tr>
<td>2. female</td>
<td>38</td>
<td>10 years</td>
<td>Unknown</td>
</tr>
<tr>
<td>3. female</td>
<td>63</td>
<td>10 years</td>
<td>PSS</td>
</tr>
<tr>
<td>4. male</td>
<td>55</td>
<td>1.5 years</td>
<td>Renal disease</td>
</tr>
<tr>
<td>5. female</td>
<td>67</td>
<td>20 years</td>
<td>SSS</td>
</tr>
<tr>
<td>6. male</td>
<td>45</td>
<td>4 months</td>
<td>Radiation treatment</td>
</tr>
<tr>
<td>7. female</td>
<td>63</td>
<td>8 months</td>
<td>Radiation treatment</td>
</tr>
<tr>
<td>8. male</td>
<td>54</td>
<td>13 months</td>
<td>Radiation treatment</td>
</tr>
<tr>
<td>9. male</td>
<td>65</td>
<td>2.5 years</td>
<td>Radiation treatment</td>
</tr>
<tr>
<td>10. female</td>
<td>66</td>
<td>2 years</td>
<td>Radiation treatment</td>
</tr>
</tbody>
</table>

PSS = Primary Sjögren’s Syndrome  
SSS = Secondary Sjögren’s Syndrome

**Saliva collection**

Paraffin-chewing stimulated saliva was collected from the patients before the beginning of the study and then after the acupuncture treatment was completed. The standardized salivary flow test procedures used at the department of Cariology were applied in this study.\(^{35}\) The patients 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 so as to consider 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, awaiting the radio immunoassay tests (RIA), that were to be carried out at a later date.

**Acupuncture treatment**

An experienced acupuncturist carried out the acupuncture sessions, and the acupuncture procedure as well as the points used for the treatment of xerostomia are also described in detail in earlier studies.\(^{36-38}\) The most frequently used points, used bilaterally and described in Table 2 are St3, St6 in the face, Li4 in the hands and St36 and Sp6 in the legs.\(^{36}\) The disposable needles used, were Chinese and made of stainless steel, Hwato 0.32 × 40 mm, and Cloud & Dragon, 0.30 × 15, 30 and 40 mm. After standard sterilizing of the site, the needles were inserted through the skin to the depth of 5 mm to 10 mm, and manipulated until the needle sensation (DeQi) was obtained. 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.\(^{37}\) When that sensation was achieved, the needles were left in situ, and not manipulated again unless a needle was displaced. All patients received a double series of acupuncture treatments: 24 sessions of 20 min each.

**Peptide analyses**

VIP-like immunoreactivity (VIP-LI) was analysed in the saliva samples collected from the subjects, using competitive radioimmunoassay (RIA).\(^{38}\) Vasoactive intestinal polypeptide (VIP-LI) was analysed using antiserum VIP2 raised against conjugated natural porcine VIP. The antiserum does not react with gastrin, pancreatic polypeptide, glucagon, NPY or neurotensin. Intra- and interassay

---

**Table 1** The patients involved in this study

<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>PSS</td>
</tr>
<tr>
<td>2. female</td>
<td>38</td>
<td>10 years</td>
<td>Unknown</td>
</tr>
<tr>
<td>3. female</td>
<td>63</td>
<td>10 years</td>
<td>PSS</td>
</tr>
<tr>
<td>4. male</td>
<td>55</td>
<td>1.5 years</td>
<td>Renal disease</td>
</tr>
<tr>
<td>5. female</td>
<td>67</td>
<td>20 years</td>
<td>SSS</td>
</tr>
<tr>
<td>6. male</td>
<td>45</td>
<td>4 months</td>
<td>Radiation treatment</td>
</tr>
<tr>
<td>7. female</td>
<td>63</td>
<td>8 months</td>
<td>Radiation treatment</td>
</tr>
<tr>
<td>8. male</td>
<td>54</td>
<td>13 months</td>
<td>Radiation treatment</td>
</tr>
<tr>
<td>9. male</td>
<td>65</td>
<td>2.5 years</td>
<td>Radiation treatment</td>
</tr>
<tr>
<td>10. female</td>
<td>66</td>
<td>2 years</td>
<td>Radiation treatment</td>
</tr>
</tbody>
</table>

PSS = Primary Sjögren’s Syndrome  
SSS = Secondary Sjögren’s Syndrome

Sensory stimulation increases the release of vasoactive intestinal polypeptide.

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>
</table>
| St 3 Juliao | When the eyes look straight forward, it is inferior to the pupilla at the level of the lower border of the alae nasi | m. levator labii sup. | n. trigeminus, r. ophthal-
|m. ophtalmicus, n. infraorbitale |
| St 6 Jiache | One finger width anterior and superior to the angle of lower jaw, at the prominence of the m. masseter during mastication | m. masseter | n. trigeminus, r. man-
dibularis | n. facialis, r. mandibularis, |
| | | | | n. trigeminus |
| Li 4 Hegu | On the middle point of os metacarpale II, on the prominence of the 1st m. interossei dorsales slightly towards the side of the index. | m. interosseus | n. radialis, |
dors. m. abduct. | (C 8, Th 1), |
pollicis | r. superficialis |
m. lumbricale I | n. medianus |
| St 36 Zusanli | 10 cm below apex patellae, one finger width lateral to the cri
ta anterior tibiae | m. tibialis ant. | n. cutaneus lat. |
lv (L 5, S 1,2) | n. peroneus |
| Sp 6 Sanyinjiao | 10 cm above the highest point of the malleolus medialis at the posterior border of the tibia | m. flexor digitorum long. | n. sapheneus |
m. tibialis post. | (L 3, 4) | n. tibialis |

The lower detection limit in all saliva samples was 0.1 fmol/mL for all peptide assessments.

High-performance liquid chromatography (HPLC): reverse-phase HPLC was applied to samples extracted with 2 M acetic acid in 4% EDTA. HPLC was performed using a Waters Delta Pac C18 300 Å, 3.9 mm × 15 cm column, eluted with a 40-min linear gradient of acetonitrile in water containing 0.1% trifluoracetic acid. Two Pharmacia P3500 pumps were controlled by Pharmacia GP250 gradient programmer. A gradient of 20–50% acetonitrile was used. Samples were passed through Millipore GS filters (0.45 μm) prior to chromatography to remove particulate matter. Samples of 200 μL were injected into columns. Fractions of 0.5 mL were collected at an elution rate of 1.0 mL/min. Each fraction was lyophilized and redisolved in 100 μL distilled water before analysis. The fractions were assayed for immunoreactivity in the tubes used for their collection.

Statistical analyses
Wilcoxon's test (paired two samples) was used in order to compare the concentrations of VIP 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
Acupuncture treatment resulted in an increase of saliva production. The concentrations of VIP in the saliva were significantly higher after the end of acupuncture treatment as compared to the base line levels (before the AP was started). The results are visualized in Figure 1 (p < 0.05). The correlation between the changes in VIP-concentration and salivary flow was 0.66.

HPLC showed a distinct immunoreactive component with regard to VIP, 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
The xerostomic patients that were treated with acupuncture showed an increase in salivary secretion rates. We found a significant increase of the concentration of VIP in the stimulated saliva of the 10 patients that were...
investigated in this study and it was correlated to the increase of the salivary secretion.

The secretion of saliva is regulated by the autonomic nervous system. It is influenced by the excitation of afferent nerve fibres from the masticatory system leading to reflex excitation of salivary parasympathetic and sympathetic activity. It has been shown that salivary neurons in the lateral reticular formation of the lower brain stem react to stimulation of afferent branches of the trigeminal. Input from group II and group III afferent fibres of the trigeminal nerve activate salivary afferent neurons, resulting in salivary secretion. 42

The connection between VIP and salivary functions has been investigated in several studies. VIP-containing nerves have been found in close association with the acini, ducts and blood vessels, 19,16 as well as in close proximity of mucous endpieces and palatal glands, intercalated ducts of the parotid gland and excretory ducts of parotid, labial and palatal glands. 17,18 Animal studies have shown that VIP can influence the saliva secretion, producing relatively small amounts of saliva, the most from gland, submandibularis, the least from gland, sublingualis, and that VIP can increase the release of proteins. Like SP and calcitonin gene-related peptide (CGRP), VIP increases significantly the blood flow in the salivary glands. Together with CGRP, VIP also enhances the salivary secretion evoked by SP and acetylcholine. 12,13,26-34,45,44 It has also been demonstrated that repeated infusions of SP and VIP are capable of preventing the expected reduction in gland weight following denervation or liquid diet. 19 Human studies have shown that VIP alongside SP is a potent vasodilator in the human submandibular gland, 14 and it has been shown that VIP potentiates the salivary response to acetylcholine. 29 The finding of neuronal VIP in the salivary glands, its release upon nerve stimulation, and its known effect on local blood flow support the view that VIP is neurotransmitter in the salivary glands. 25,26

Apart from the secretory and vasomotor influence of VIP, it has been suggested that this neuropeptide can also function as a growth factor in wound healing and angiogenesis, and that it has a trophic influence on the salivary gland tissue. 24,45 There are also suggestions that immune cells as well as nerve cells can synthesize VIP, 16 and that there are specific receptors; VIP-R1 and VIP-R2 on human T-cell that bind VIP. 47,48 The immunoregulatory effect of VIP's immune response seems to be the inhibition of lymphocyte migration by way of modulating their adherence to the vascular endothelium. 49 VIP exerts its anti-inflammatory effect by downregulating the immune response when the inflammation has acted out its role. 23

Several previous and ongoing investigations have shown that acupuncture can improve the salivary secretion of xerostomic patients and that the increased salivary flow rates persist during a long observation period. 5-24 It is of utmost importance, however, to elucidate the mechanisms behind the effect of acupuncture on salivary gland function in order to achieve a scientifically acceptable explanation of the efficacy of this treatment. Otherwise there are only a few ways of alleviating xerostomia, none of them very effective or capable of producing any long-lasting results. 50-53

Acupuncture has been used in China for treatment of diseases and their symptoms for more than 5000 years. The vast empiric knowledge that has been accumulated during the ages has propelled this method of treatment into the western medical world, and although it is still regarded with healthy scepticism, acupuncture is widely used. Many scientific studies have been conducted during the recent decades in order to elucidate the mechanisms behind the effects of acupuncture, as traditional Chinese philosophy is unacceptable to the western scientific community. The results of these numerous studies have led to a better understanding of the function of sensory stimulation as well as to an acceptance of acupuncture as a therapeutic method in the treatment of different illnesses and acute pains. As more clinical investigations limit or broaden the field of the use of acupuncture, its mechanisms, when found effective, must be investigated in each instance, in order to provide a scientific basis for its application.

The investigations of acupuncture mechanisms have revealed that acupuncture stimulation leads to a release of endogenous opioids and oxytocin. The endorphins, especially β-endorphin, bind to opioid receptors, affecting blood pressure and body temperature as well as
exerting pain control by their affinity to \( \mu \)-receptors. The pain sensitivity and autonomic functions are influenced on the central level, through the hypothalamus, midbrain and brainstem nuclei. It has been suggested that VIP-release due to sensory stimulation was the agent responsible for the effect of acupuncture-like stimuli that caused an increase of temperature and microcirculation in the skin. The influence of acupuncture on salivary secretion might be mediated via the parasympathetic nervous system as reflexive response to sensory stimulation of group III afferent A-delta nerve fibres and possibly C-fibres of n. trigeminus, leading to activation of the salivary neurons in the lateral reticular formation of the lower brain stem.

We concluded that one of the mechanisms through which the acupuncture stimulation can lead to an increase of the salivary flow rates in xerostomic patients might be the release of VIP. The anti-inflammatory properties of VIP, together with its role as growth factor could, aside from its secretory influence, provide an explanation of the long-term positive effects of acupuncture treatment.

The Swedish Dental Society, the Swedish Patent Revenue Research Fund, the Gustav Vth 80-year Anniversary Foundation, the Professor Nanna Schwartz Foundation and the Swedish Society against Rheumatism (RMR) supported this study.

REFERENCES

33. Iwabuchi Y, Masuhara T. Effects of vasoactive intestinal peptide and its homologues on the substance P-mediated secretion of...