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Theophylline increases saliva sonic hedgehog and improves taste dysfunction

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ABSTRACT

Objective: To determine changes in saliva sonic hedgehog (Shh) and in taste dysfunction before and after oral theophylline treatment.

Design: Shh was measured in parotid saliva of both normal subjects and patients with taste dysfunction of multiple etiologies by use of a sensitive spectrophotometric ELISA assay. Taste dysfunction was defined clinically by both subjective inhibition of taste function (including acuity loss) and impaired gustometry.

Results: Theophylline treatment increased patient saliva Shh and improved taste dysfunction both subjectively and by gustometry.

Conclusions: By use of these systematic clinical measurements theophylline can be demonstrated to increase saliva Shh and improve taste dysfunction. These results are consistent with saliva Shh acting as a taste bud growth factor which stimulates stem cells of taste buds to initiate development and perpetuation of taste bud receptors. Measurements of saliva Shh provide an important marker for the presence of taste dysfunction and in the improvement by theophylline treatment.

1. Introduction

We have previously demonstrated the presence of sonic hedgehog (Shh) in parotid saliva and its decrease in patients with taste dysfunction (Henkin, Kn & ppel, Abdelmeguid, Stateman, & Hosein, 2016). Shh is a protein which is well known to regulate morphogenesis including in the taste system. We also previously demonstrated that Shh was decreased in nasal mucus of patients with smell dysfunction (Henkin, Hosein, Stateman, & Kn & ppel, 2016) and that treatment with oral theophylline increased nasal mucus Shh associated with improvement in smell dysfunction (Henkin, Hosein, Stateman, Kn & ppel, & Abdelmeguid, 2016). Theophylline has been shown to increase several moieties in nasal mucus considered to be growth factors in olfactory function (Henkin, 2011). These increases are associated with improved smell function. Since theophylline increased nasal mucus Shh and -improved smell dysfunction we wondered if oral theophylline-treatment might also increase saliva Shh with an associated improvement of taste dysfunction. This latter hypothesis is the subject matter of this study.

2. Methods

2.1. Subjects

2.1.1. Normal subjects

Twenty-seven volunteers, aged 22–84 y, 54 ± 5 y (Mean ± SEM) with normal taste function were studied. These volunteers were either patients who presented to The Taste and Smell Clinic in Washington, DC for evaluation of symptoms unrelated to taste loss (hypogeusia) or who were employees of The Taste and Smell Clinic who volunteered for the study. Subjects were selected in a consecutive manner and included all subjects who volunteered for the study.

2.1.2. Patients

Eighty-one patients, aged 10–88 y, 56 ± 3 y who presented to The Taste and Smell Clinic in Washington, DC for evaluation and treatment of taste and smell loss were also studied. Patients were all patients evaluated consecutively at The Clinic from 2012 to 2013. Patients were 41 men, aged 12–88 y, 54 ± 4 y and 40 women, aged 10–84 y, 51 ± 5 y. Taste dysfunction was caused by seven pathological events including post-influenza-like hypogeusia (Henkin, Larson, & Powell,

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1975) (27 patients), allergic rhinitis (Church, Bauer, Bellanti, Satterly, & Henkin, 1978) (26 patients), congenital loss of smell with associated hypogeusia (Henkin & Levy, 2002) (10 patients), head injury (Schechter & Henkin, 1974) (14 patients), post general anesthesia (Henkin, 1995) (two patients), dysgeusia and oropyrosis (Henkin, Gouliouk, & Fordyce, 2012) (one patient) and post systemic radiation (Mossman & Henkin, 1978) (one patient). Patients exhibited taste dysfunction as measured by subjective statement of acuity loss (Henkin, Levy, & Fordyce, 2013) and by impaired gustometry (Henkin et al., 2013), as previously described.

Subjective statements of acuity loss were quantitated by use of a scale from 0 to 100 (in percent) with 100 reflecting total loss of taste function, 0 reflecting no loss and a number between 0 and 100 reflecting relative degree of loss. Mean ± SEM of loss degree was measured among all patients and each pathology initiating taste dysfunction.

Gustometry measurements were obtained to evaluate taste dysfunction using a standard three stimuli forced choice staircase technique(Henkin et al., 2013). Measurements of detection (DT) and recognition (RT) thresholds, magnitude estimation (ME) and hedonics (H) for four tastants [NaCl (salt), sucrose (sweet), HCl (sour) and urea (bitter) (Henkin et al., 2013)] were obtained. Detection thresholds were defined as the least concentration of a tastant detected by the subject as different from two drops of water. Recognition thresholds were defined as the least concentration of a tastant recognized as that tastant (e.g., NaCl as salty, sucrose as sweet, etc.). Magnitude estimation was defined as the quantitative estimation of the intensity of salt, sweet, sour and bitter tastants. ME was measured on a 1–100% scale with patients judging taste intensity with respect to tastant intensity experienced prior to onset of taste dysfunction (Henkin et al., 2013). Abnormalities of taste function were measured by increased DT or RT above normal (decreased sensitivity) and/or decreased ME (decreased sensitivity) for one or more of the tastants presented (Henkin et al., 2013). H was measured as a percentage of hedonic quality of the salt, sweet, sour or bitter content of each tastant used to measure taste function. H varied with respect to perception of pleasantness, unpleasantness or neutrality for each tastant measured on a 1–100% scale with +1 – + 100 indicating pleasantness, −1 – −100 indicating unpleasantness and 0 indicating neutrality (Henkin et al., 2013).

Theophylline was administered orally in two divided doses for periods of 2–6 months with doses varying from 200 mg to 1000 mg daily, as previously described (Henkin et al., 2013). At termination of each treatment period saliva Shh, serum theophylline and taste function were measured (Henkin et al., 2013). If patients exhibited improvement in taste function they were maintained on that dose. If patients did not exhibit improvement in taste function their oral dose was increased by 200 mg and studied again after a period of 2–6 months. At the end of this period saliva Shh, serum theophylline and taste function were measured. If patients exhibited improvement in taste function they were maintained on this dose. If they did not improve treatment dose was increased by 200 mg and the process was continued until the highest dose of 1000 mg was obtained.

Study protocol was approved by the Chesapeake Institute Review Board. Each patient and subject agreed to participate in the study and signed an informed consent participation form. All subjects under age 18 y entered into the study after a parent gave informed consent.

3. Methods

Patients and volunteers collected saliva by placement of a Lashley cup over Stensen's duct of one parotid gland with saliva stimulated by lingual placement of concentrated lemon juice (Henkin et al., 1978; Henkin, Lippoldt et al., 1975). Saliva was collected in plastic tubes in ice for timed periods of 8–10 min, as previously described (Henkin et al., 1978). Flow rate was measured by mean flow over a four minute time period, as previously described (Henkin et al., 1978; Henkin, Lippoldt et al., 1975). Samples were stored at −20 °C until analyzed.

Each sample was analyzed by use of a sensitive spectrophotometric ELISA technique obtained from Abcam Inc. (Cambridge, MA) for the analysis of Shh. Analysis of duplicate samples agreed within 5%. All analyses were made independent of the knowledge of the status of any subject. Only after all samples were analyzed were results tabulated and samples classified in relationship to subject status. Serum theophylline was measured by a standard fluorescent assay (Li, Benovic, Buckler, & Burd, 1981).

Results were not formulated until all results were analyzed. Results were then calculated with respect to before and after theophylline treatment and analyzed such that mean ± SEM levels in each category were obtained and results compared using Student *t*-tests with $p < 0.05$ considered significant. Pearson product-moment correlation analyses were employed with $p < 0.05$ considered significant. ANOVA analyses were also used with $p < 0.05$ considered significant.

4. Results

Changes in saliva Shh in patients with taste dysfunction before and after treatment with theophylline compared to levels in normal subjects are shown in Table 1. Results indicate that patients' saliva Shh levels before treatment were significantly lower than in normal subjects. After theophylline treatment Shh levels increased significantly to levels similar to those measured in normal subjects (Table 1). Saliva Shh levels increased significantly in both men and women (Table 1). There were no significant differences in saliva Shh levels between men and women patients with taste dysfunction (Table 1).

Saliva Shh levels in patients with taste dysfunction related to several pathological causes before and after theophylline treatment are shown in Table 2. Saliva Shh levels in all patients were decreased below normal values prior to treatment but saliva Shh increased in all patients after theophylline treatment with levels increasing the most in patients after a post-influenza-like infection and in patients with head injury. Saliva Shh levels increased the least among patients with allergic rhinitis.

Subjective changes in taste dysfunction for both acuity and flavor loss are shown in Table 3. Improvement in taste acuity and in flavor perception was reported in 58% of patients after theophylline treatment with a mean improvement in sensitivity of 28% and 24%, respectively (Table 3).

Changes in saliva Shh and in gustometry in all patients before and after theophylline treatment are shown in Table 4. Results indicate that saliva Shh levels increased significantly after theophylline treatment. Results also indicate that measurements of DT, RT and ME increased in sensitivity after theophylline treatment for all tastants. However, DTs decreased significantly (increased sensitivity) for sucrose and urea and RTs decreased significantly (increased sensitivity) for all tastants. Hs increased in pleasantness for sucrose and increased in unpleasantness for HCl and urea.

Changes in saliva Shh in men and women patients with taste

Table 1
Saliva sonic hedgehog in patients with taste dysfunction before and after treatment with oral theophylline.

Patients	Saliva sonic hedgehog (pmol/ml)
Before treatment (81)	71 ± 4[+]
After treatment (81)	199 ± 20[a]
Men (41)	180 ± 15[a]
Women (40)	213 ± 33[a]
Normal subjects (27)	184 ± 12

() Patient number.
[+] Mean ± SEM.
With respect to untreated patients.
[a] $p < 0.001$.