Gustin concentration changes relative to salivary zinc and taste in humans

(zinc deficiency/zinc treatment/taste dysfunction/salivary proteins/parotid saliva)

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ABSTRACT Biochemical characteristics of gustin, the major zinc protein in human parotid saliva, are similar whether the protein is isolated from subjects with normal taste acuity or from patients with hypogeusia (who may have as little as 1/5th as much parotid saliva gustin as normal subjects do). Zinc concentration in fraction II of parotid saliva, the fraction in which gustin is found on Sephadex G-150 or Sephacryl S-200 column chromatography, is proportional to the gustin content of saliva and is decreased in patients with lower than normal total parotid saliva zinc. The quantity and spectrophotometric indices of all other protein fractions isolated from patients by these column chromatographic techniques did not differ from those of normals. One patient with proven hypogeusia and low concentrations of zinc in total parotid saliva and fraction II, after 9 days of treatment with exogenous zinc, showed a 150% increase in fraction II zinc and a concomitant increase in apparent gustin levels; these changes preceded the return of normal taste function. These data demonstrate that zinc treatment can affect both taste and gustin concentrations in hypogeusia.

Gustin is the major zinc-containing protein in human parotid saliva, and it makes up 3% of the total protein (1). It has a molecular weight of 37,000, is composed of 8% histidine residues, and contains 1 mol of zinc per mol of protein (1); in the presence of excess zinc a second mole of zinc can be loosely bound; this zinc can be replaced by several other metal cations (2). Gustin is found in fraction II of the six fractions into which human parotid saliva is separated by Sephadex G-150 or Sephacryl S-200 column chromatography. Zinc in fraction II is 75–80% of the total parotid saliva zinc (1), is associated exclusively with the gustin found in this fraction, and is therefore a useful marker to identify gustin. Two optical characteristics, A_{280} and fluorescence, are also useful to identify gustin in this fraction (1, 3). Although its specific functions have not been well documented, gustin has been implicated with the growth and development of taste buds, and its functional similarity to nerve growth factor has been noted (4, 5). Its relative absence in patients with decreased taste acuity (hypogeusia), associated with low levels of total parotid saliva zinc (6), pathological changes in taste buds (7, 8), and specific displacement of mouse nerve growth factor from isolated, purified bovine taste bud membranes (4, 5), have been used to support these hypotheses of function. Treatment with zinc in some patients with hypogeusia has resulted in the return of normal taste function (9–12) along with normalization of taste bud architecture (8) and salivary zinc levels (7, 9, 12).

Proteins in other column chromatographic fractions of human parotid saliva have not been fully characterized, but these fractions have been defined on the basis of optical characteristics (1, 3). Fraction I was characterized by high relative fluores-

cence, fraction V by relatively high levels of protein without zinc or fluorescence, and fraction VI by two major peaks, the first characterized by high relative fluorescence, the second, primarily by absorbance at 280 nm. Fraction II was characterized by a high level of protein and zinc, the major protein being a carbohydrate-containing protein previously noted by others (13–18). This glycoprotein constitutes 92% of the protein in this fraction (3); 80% of its amino acid composition is proline, glycine, and glutamic acid, and it is essentially devoid of aromatic amino acids; it stains pink-violet with Coomassie brilliant blue R250 after gel electrophoresis; and it has a molecular weight of approximately 34,000 (3). This glycoprotein is, in reality, a family of glycoproteins; it is also found in smaller amounts in fractions III and V, and it makes up about 75–80% of the total parotid saliva protein. Physiologically, it appears to be bound to gustin in parotid saliva (2), suggesting that this complex may be the functional active form for taste bud growth and nutrition.

Although low concentrations of saliva zinc have been measured in some patients with hypogeusia (6, 19), associated changes in gustin have not been demonstrated. Furthermore, although zinc administration has been associated with increased salivary zinc content and correction of taste loss in some patients with hypogeusia (9–12), associated changes in gustin have not been demonstrated.

It is the purpose of the present study to investigate changes in the biosynthesis of gustin in patients with hypogeusia and to attempt to correlate the effects of zinc treatment on both gustin levels and taste function.

MATERIALS AND METHODS

Subjects were 16 volunteers with normal taste acuity and 4 patients (A, B, C, D) with hypogeusia (7). Parotid salivary proteins and taste function were studied in patient D before and after daily treatment with 100 mg of exogenous zinc ion, as zinc sulfate, for 14 consecutive days.

Parotid saliva was collected by using plastic Lashley cups placed over Stenson's ducts on both sides; flow was stimulated by placing reconstituted lemon juice (Borden, Columbus, OH) in the oral cavity (1). In this manner, 100–150 ml of whole parotid saliva was collected in 60–90 min.

All glassware and reagents were essentially zinc free (1, 3) as determined by flame aspiration atomic absorption spectrophotometry. When necessary, solutions were passed through columns containing Chelex 100 (Bio-Rad), which reduced zinc content to 10–20 nM. Plastic equipment was used whenever possible.

Metal concentrations were determined by flame aspiration atomic absorption spectrophotometry on an IL 251 spectrophotometer (Instrumentation Laboratory, Lexington, MA) except in patient D. For this patient, zinc was measured in an IL

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Abbreviation: ppb, parts per billion (10^9).

355 flameless atomizer coupled to an IL 153 atomic absorption spectrophotometer. Comparative measurements between the earlier flameless and the presently used flame aspiration technique indicate that earlier values were approximately 30% of those presently obtained, due primarily to a constant and uniform loss of zinc from the open tantalum ribbon of the flameless atomizer during the ashing process.

Gustin was purified from saliva as previously described by chromatography on Sephacryl S-200 (2) or, for patient D (1), on Sephadex G-150, followed by chromatography on DEAE-Sephadex A-50 and finally by chromatography on CM-cellulose (1). Protein characteristics of the effluent from each column were determined by several optical techniques (1). Fluorescence (F ; excitation at 280 nm, emission at 340 nm) was measured on a Perkin-Elmer MPF 44A fluorimeter, with *N*-acetyltryptophan amide (A_{280} of 0.05 calibrated to 50 F units) as a standard. Absorbance at 280 nm was measured on a Beckman DU 2 spectrophotometer as was $\Delta 215$ (absorbance at 215 nm minus absorbance at 225 nm). Protein concentration was determined by calculations based upon $\Delta 215$ (1, 20) and by the method of Lowry (21). Purity of gustin was determined by the ratios of zinc, F , A_{280} , and $\Delta 215$ and by gel electrophoresis with sodium dodecyl sulfate of Tris/glycine, as reported (1).

Taste function was determined by measurements of detection and recognition thresholds [by a standard forced-choice three-stimulus drop technique for representatives of four taste qualities (NaCl, salt; sucrose, sweet; HCl, sour; urea, bitter) (7, 12)] and by suprathreshold magnitude estimation measurements for each taste quality [by a standard technique in which intensity of each appropriately recognized tastant was estimated on a scale from 1 to 100, all correct responses were averaged, and this value (in %) and the SEM are reported for each taste quality (12)]. Hypogeusia was considered present if detection or recognition thresholds for any taste quality was above the upper limit of the normal range (12) or if forced magnitude estimation was significantly increased above normal.

RESULTS

Comparison of spectrophotometric properties of the chromatographed parotid saliva proteins from the six normal subjects and three patients (A, B, C) with hypogeusia indicates that proteins in fractions I, III, IV, V, and VI are present in similar quantities in both groups (Table 1). However, spectrophotometric measurements of fraction II, which contains by glycoproteins and gustin, differ significantly (Fig. 1). Fraction II from the patients has significantly less A_{280} (63%), F (46%), and zinc (73%) than fraction II from normal subjects, although the total protein content is approximately the same (Fig. 1). Because the glycoproteins make up the major part of this fraction's total protein and contain essentially no aromatic amino acids and no zinc, the changes observed in fraction II may be related almost exclusively to decreases in gustin.

To determine if the observed changes were directly related to changes in gustin, fraction II proteins from the patients and the normal subjects were chromatographed on DEAE-Sephadex A-50 and CM-cellulose columns to obtain purified gustin. Gustin isolated from patients and normal subjects was identical with respect to its $Zn/\Delta 215$ and Zn/A_{280} ratios and electrophoretic mobility (Table 2), although F in the patients was approximately 1/3rd less (Fig. 2). The decreased F did not correspond to any observable change in secondary structure (2) but may be attributable to difficulties in comparing relative F measurements. Similarities in chromatographic behavior, electrophoresis, spectrophotometric indices, and ratios between zinc and each protein spectrophotometric property (Table 2) indicate that the large decreases in A_{280} , F , and zinc in fraction II (Fig.

Table 1. Spectrophotometric properties of salivary proteins in normal subjects and in patients with hypogeusia

Fraction	A_{280}	$\Delta 215$	F	Zn	
Normal Subjects ($n = 6$)					
I	0.46 ± 0.11	3.6 ± 0.3	$840 \pm$	55	67
II	See Fig. 1				
III	0.53 ± 0.04	115.2 ± 12.0	$1,140 \pm$	147	106
IV	0.36 ± 0.05	58.6 ± 5.3	$144 \pm$	11	39
V	0.64 ± 0.09	91.2 ± 5.1	$388 \pm$	26	42
VI	17.76 ± 1.02	59.6 ± 6.7	$161,830 \pm$	27,900	21
Patients with hypogeusia ($n = 3$)					
I	0.49 ± 0.35	0.2 ± 0.4	$720 \pm$	55	86
II	See Fig. 1				
III	0.55 ± 0.32	113 ± 20.8	$1349 \pm$	277	40
IV	0.21 ± 0.04	43.8 ± 9.6	$88 \pm$	18	27
V	0.73 ± 0.15	101.6 ± 13.3	$412 \pm$	34	18
VI	19.9 ± 1.04	61.4 ± 8.0	$146,000 \pm$	33,300	29

Data are presented as mean $\pm$ SEM of the areas under chromatographic curves of each property found for each fraction; A_{280} and $\Delta 215$ were measured as absorbance; F , by arbitrary optical units; Zn, by flame aspiration atomic absorption spectrophotometry, in ppb.

1) are not due to physical changes in gustin itself. The only major difference discovered was the decreased fraction II gustin content of the patients (0.28 mg/dl of saliva) compared to that of normal subjects (1.40 mg/dl) (Fig. 2). This reflects a 75% decrease in saliva gustin content in the patients, which is in general agreement with the 73% decrease observed in their fraction II zinc (Fig. 1).

Comparison of fraction II zinc (Fig. 1) with the actual amount of gustin isolated from each subject (Table 1) reveals a direct relationship between these two indices. For each mg of gustin in fraction II, Zn is present at approximately 1000 parts per billion (ppb; i.e., 1 in 10^9). In contrast, saliva gustin concentration cannot be estimated accurately from whole parotid saliva

FIG. 1. Comparison of spectrophotometric properties of parotid saliva proteins from fraction II of Sephacryl S-200 chromatography in normal subjects and in patients with hypogeusia. Total $\Delta 215$ (sum of $\Delta 215$ from all fractions) is presented for comparison. The mean values determined for six normal subjects are expressed as 100%. The mean values determined for three patients with hypogeusia are expressed as percent of normal; lines above bars are relative SD. Numbers above bars indicate absolute (see Table 1) mean values $\pm$ SD. *, $P < 0.01$; **, $P < 0.05$ with respect to normals.

Table 2. Ratios of several spectrophotometric properties and zinc in gustin purified from subjects with normal taste function and from patients with hypogeusia

Gustin source	mg gustin dl saliva*	A_{280} $\Delta 215^\dagger$	F $\Delta 215^\dagger$	Zn $\Delta 215^\dagger$
Normal subjects (n = 6)	1.40 ± 0.18	0.30	18.7	248
Patients with hypogeusia (n = 3)	0.28 ± 0.03‡	0.30	16.8	300

* Mean ± SEM of values obtained after complete purification of gustin from parotid saliva.

† Values obtained from gustin after final purification by CM-cellulose chromatography.

‡ $P < 0.01$ with respect to normals.

zinc levels; while the average saliva zinc concentration from normal subjects is about twice that of the patients (88 ± 9 ppb vs. 37 ± 5 ppb, respectively), gustin concentrations in patients may be as low as 1/5th that of normal subjects (Fig. 2).

The glycoproteins from fraction II were similar in normal subjects and in patients. They also were found to be similar in composition as indicated by similarities in carbohydrate content, amino acid composition, electrophoretic mobility, and pink-violet staining with Coomassie brilliant blue R250 (2) after gel chromatography.

Fraction II zinc content and other characteristics were followed in patient D before and after zinc therapy. After zinc treatment, fraction II from the patient's saliva exhibited significant increases in zinc and F but not in $\Delta 215$ (Fig. 3). Fraction II zinc increased from a mean of 95 ppb before treatment to 144 ppb after 3 days of zinc therapy (Fig. 3); fraction II zinc continued to rise through the 9th day and then decreased slightly to a final value of 153% over pretreatment levels after 14 days of therapy.

FIG. 2. Comparison of spectrophotometric properties of purified gustin isolated from parotid saliva of six subjects with normal taste function (set as 100%) and three patients with hypogeusia; lines above bars indicate relative SD. Numbers above bars indicate absolute mean values ± SD. *, $P < 0.01$ with respect to normals.

FIG. 3. Zinc, $\Delta 215$, and F in fraction II in parotid saliva from patient D before (Pre) and during treatment with 100 mg of exogenous zinc ion, as $ZnSO_4$, orally, daily for 14 days. Values for $\Delta 215$ and F were obtained from chromatography of parotid saliva on Sephadex G-150 and represent the area under the curve of each fraction II property. Pretreatment values of zinc and F shown on the left (mean ± SD) are from four separate chromatograms from patient D. Zinc concentrations are lower than those in Figs. 1 and 2 due to measurement on a tantalum ribbon (see Materials and Methods).

In association with the presumed increase in gustin, a concomitant increase in this patient's taste acuity occurred (Fig. 4). Before zinc therapy, detection and recognition thresholds for HCl and urea were impaired, although thresholds for NaCl and sucrose were at the upper limit of normal (Fig. 4A); magnitude estimation for NaCl, HCl, and urea was also below normal (Fig. 4B). After 6 days of zinc therapy, after fraction II zinc had increased by 50%, detection and recognition thresholds for urea, although improved, were still above the upper limit of normal; detection threshold for HCl decreased into the normal range, although the ability to recognize this tastant as sour was still impaired. At this time magnitude estimation for all tastants increased to within the normal range, where they remained throughout the treatment period, although continuing to improve until day 9 of therapy. After 9 days of therapy, fraction II zinc reached its maximal value and detection and recognition thresholds for all tastants were within the normal range; thresholds for all tastants continued to improve until day 12 of therapy (Fig. 4A). The time at which the maximal changes in taste thresholds occurred was on day 12 (129%), 3 days after the maximal increase in both fraction II zinc (160%) and the Zn/ F ratio (70%) occurred (Fig. 3). The time at which maximal change in magnitude estimation (171%) occurred was on day 9 (Fig. 4B), 3 days before the maximal changes in taste thresholds, and coincided with the maximal changes in fraction II zinc and Zn/ F (Fig. 3). After the discontinuation of zinc treatment, threshold values for urea worsened within 7 days to levels similar to those measured prior to zinc treatment; total parotid saliva zinc levels fell from 75 to 39 ppb.

DISCUSSION

These studies indicate that decreased gustin concentration is the major change that occurs in parotid saliva of patients with hypogeusia and low parotid saliva zinc level, the decrease being to as little as 20% of the concentration in normal subjects. This decrease is manifested by decreased A_{280} , zinc, and F in fraction II, but not total protein (i.e., the 92% composed of proline/glycine/glutamic acid-containing glycoproteins, which remains constant).

Treatment of a patient with hypogeusia with zinc resulted in a rapid increase in total saliva zinc and a concomitant increase

FIG. 4. Changes in taste acuity in patient D before and during treatment with exogenous oral zinc. (A) Taste detection and recognition thresholds for each of four taste qualities (salt, sweet, sour, and bitter, from left to right) in bottle units (BU) have been summed; the upper limit of normal for each taste quality is 8. The large number represents the sum of all four tastants, the upper limit of normal being 32. As treatment proceeded taste function improved as noted by decreased thresholds. (B) Taste forced scaling (magnitude estimation). Measurements were taken at the same time as the thresholds were determined. As treatment proceeded, forced scaling, improved, as noted by increased values. ●, NaCl; □, sucrose; △, HCl; ○, urea.

in fraction II F and zinc, but not $\Delta 215$, while these characteristics in all other salivary fractions remained constant. During zinc treatment, fraction II F increased 44%, similar to the amount observed to be decreased in fraction II in hypogeusia patients prior to treatment; gustin levels also appeared to increase proportionately with fraction II zinc. These results suggest that gustin biosynthesis is induced by zinc in much the same manner as metallothionein is induced by Zn, Cd, or Cu (22–26) or ferritin is induced by Fe (27). Apogustin has not been detected in parotid saliva of patients with hypogeusia, but a concerted search for this substance has not been carried out. It is conceivable that in zinc deficiency an unstable apogustin may be synthesized and rapidly turned over, in which case zinc could act to stabilize the protein rather than induce its synthesis. Metals have been shown to be necessary to maintain conformational stability of some metalloproteins (28, 29). Recent work also indicates that apometallothionein is rapidly turned over, whereas holometallothionein is relatively long lived (30).

The decreased gustin levels may also relate to an inability to absorb, transport, or store zinc. Approximately one-quarter of patients with taste and smell dysfunction have been found to exhibit zinc malabsorption (31), and patients with chronic renal disease exhibit hypogeusia, hypogonadism, and other signs of zinc deficiency (32–38), which have been corrected with exogenous zinc therapy in controlled clinical trials (36–38).

Although the detailed case study of only one patient treated with zinc has been included here, the characteristics of the changes in saliva, clinical symptoms, and taste responsiveness to therapy are representative of a number of such patients. It is also important to point out that many patients with hypogeusia do not exhibit zinc deficiency, that their taste dysfunction can occur without alterations in either salivary zinc or gustin, that treatment with zinc can be ineffective, and that treatment with a placebo has been useful in correcting hypogeusia (12).

The changes observed in taste function followed the changes observed in salivary gustin. The return of magnitude estimation to normal before taste thresholds is consistent with the hypothesis that this function depends upon the interaction of large numbers of taste buds rather than upon the normal function of individual taste buds (12, 39). While not all taste buds had presumably returned to normal function in response to higher gus-

tin levels, enough recovery may have occurred to allow for the achievement of normal magnitude estimation, consistent with the known rapid turnover rate of taste buds in mammals (40) and with the hypothesis that gustin acts as a taste bud growth factor. The last taste qualities to return to normal were those of sour and bitter; fewer taste buds are devoted to the detection and recognition of sour and bitter tastants (12), and these taste buds are most easily affected by pathological conditions (11, 41).

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