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SHORT COMMUNICATION

FREE AMINO ACIDS IN HUMAN PALATINE GLAND SECRETIONS

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Summary—Human palatine gland saliva (HPS) was collected from young adult subjects (mean age 22 yr) of both sexes by gentle mechanical stimulation. The HPS contained 9–13 of the common amino acids within the range of 2–850 nmol/ml, but most samples had low or no concentrations of arginine, tryptophan and proline. Methionine and cysteine were not reliably detected. The molar ratios of most amino acids varied between subjects, but the ratio of leucine to isoleucine was remarkably constant (mean ± SD = 1.86 ± 0.28; $n = 28$). The minimum average secretion rate of HPS, using gentle mechanical stimulation, was estimated to be about 25 mg/min (wet weight).

The secretions produced from the human minor salivary glands may play a role in the formation of the acquired pellicle and dental plaque (Hensten-Pettersen, 1975, 1979), as well as lubricating the oral epithelium. There is evidence that mucins from these glands participate in non-immune protection of the mouth (Tabak *et al.*, 1982). In this respect the role of the numerous minor salivary glands in mucin production is central: these glands may contribute up to 70% of the total oral mucin production, although their contribution to the total, daily salivary volume is modest, about 6–10% (Dawes and Wood, 1973a; Milne and Dawes, 1973).

Human palatine gland secretions (HPS) contain mucosubstances, albumin-like proteins, secretory IgA, blood-group substances, virus-haemaglutination activity, α-amylase, and some kallikrein activity, but no lysozyme (Hensten-Pettersen, 1975, 1976; Hensten-Pettersen and Sonju, 1975; Hensten-Pettersen and Jacobsen, 1975) and peroxidase (Mäkinen *et al.*, 1983). There seems to be agreement upon the absence of lysozyme in HPS, but disagreement concerning the presence of α-amylase (Hensten-Pettersen, 1975; Mäkinen *et al.*, 1983), which is virtually absent in human labial gland secretions (Dawes and Wood, 1973b). HPS contains proteolytic activity (Mäkinen *et al.*, 1983, 1985a, 1985b). Others have elucidated the sodium and potassium content of HPS (Kaaber, 1977), and various chromatographic procedures have been used to evaluate HPS proteins (Shiba *et al.*, 1980, 1983). We have now examined the occurrence of free amino acids in HPS.

HPS samples were collected from 36 21–23 yr old white dental students of both sexes. The samples were obtained at 008–0010 h after normal breakfast and oral hygiene procedures. The cleaning of the palate and the mechanical stimulation of the hard palate (with a round-ended instrument) were as described by Mäkinen *et al.* (1985b), except that the appearing HPS was absorbed into 6 × 15 mm preweighed, sterilized strips of a Cambrelle absorbent polymer (Camtex Fabrics Ltd, Lilleyhall, Workington, Cumbria, England). The strips were handled with forceps. Regardless of differences in secretion rates, all HPS appeared as small distinct drops on the hard palate, which were collected during a standard 5-min period. The weight of the used strips was immediately determined, after which they were immersed in 0.5 ml of water in small, tightly capped plastic vials, and these were stored at −22°C prior to analyses. After thawing, the polymer strips containing the HPS were inserted along with the unabsorbed water into Centricon-10 concentration tubes (nominal cut-off 10000 mol. wt; Amicon, Danvers, Mass., U.S.A.) The tubes were centrifuged gently for 2–3 min (1000 *g*) to obtain an 0.2–0.3 ml sample of essentially protein-free HPS. During this procedure virtually all visible liquid had passed through the filter. These filtrates were analysed for free amino acids. Unused polymer strips were treated with water as above. A second lot of HPS was collected by a capillary method (Mäkinen *et al.*, 1983).

The aqueous HPS extracts from crushed glass capillaries and polymer strips were used to determine the free amino acids of HPS. The amino acid concentrations were determined using a Beckman Model 6300 Automated Amino Acid Analyser according to standard procedures (ninhydrin detection; programme for physiological fluids). Norleucine was used as an internal standard.

By wet weight the polymer strips obtained from female ($n = 19$) and male ($n = 17$) subjects contained 128.5 ± 137.7 and 121.5 ± 154.7 mg HPS, respectively (mean ± SD). The ranges were 3.7–325 mg and 4.2–603 mg, respectively. Thus, a rough estimate of

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Table 1. Concentration of free amino acids in human palatine gland secretions

Amino acid	Concentration (nmol ml^{-1})	
	Mean ± SD	Range†
Aspartic acid	31 ± 73 (31)*	11–125
Serine	225 ± 207 (34)	23–850
Glutamic acid	157 ± 91 (34)	52–418
Glycine	103 ± 70 (34)	26–310
Alanine	130 ± 108 (34)	29–415
Valine	106 ± 106 (34)	22–227
Isoleucine	40 ± 35 (31)	4–131
Leucine	68 ± 56 (31)	14–236
Tyrosine	62 ± 48 (21)	15–234
Phenylalanine	53 ± 30 (12)	20–125
Histidine	17 ± 18 (16)	2–83
Lysine	62 ± 43 (34)	17–178

*The values in parentheses show the number of individual HPS samples exhibiting an amino acid concentration exceeding the detection limit of 0.5 nmol ml^{-1}.

†Some samples may have contained less than 0.5 nmol ml^{-1} of amino acids not indicated here.

the secretion rate of HPS from the hard palate of these female and male subjects is about 26 and 24 mg/min, respectively.

The specific gravity of HPS (determined with glass capillaries) approximated to 1.0, admitting the use of sample weights to express the free amino acid content in nmol/ml. The free amino acid levels of HPS in the Centricon filtrates are shown in Table 1. Only those amino acids that consistently occurred in most samples, and whose levels exceeded the 0.5 nmol/ml detection limit set for the amino acid analyser under our conditions, were included in calculations. None of the samples contained measurable amounts of cysteine and arginine. Some amino acids were detected only in a few samples: a ninhydrin-positive compound with the same retention time as proline occurred in two samples out of 36 (44 ± 30 nmol/ml; mean ± SD); a compound with identical retention time of threonine was found in four samples (23 ± 25 nmol/ml), and also to that of tryptophan (14 ± 7 nmol/ml) in four samples. We were unable to measure reliably free methionine in HPS. All samples contained traces of hydroxyproline and phosphoserine and other compounds normally appearing in the most acidic region (taurine, phosphoethanolamine and urea): these were not quantitated but identified qualitatively. All samples contained large amounts of uric acid. Asparagine and glutamine were present in all samples, but the overlapping of these amino acids with other ninhydrin-positive compounds did not allow reliable quantitative analysis. Furthermore, it was not known to what extent possible deaminase enzymes present in HPS would have affected the levels of these compounds.

In spite of large variations in secretion rates of HPS and in the quantities of HPS obtained, the molar ratio of leucine to isoleucine was strikingly similar in all samples, approximating to 1.86 ± 0.28 (mean ± SD; $n = 28$). The molar ratio of glutamic acid to aspartic acid, for example, varied to a much higher extent (i.e. 4.64 ± 1.95; $n = 33$). The overall free amino acid pool of HPS was characterized by the presence of acidic amino acids and those with hydrophobic or hydroxylic side-chains. Smaller HPS samples (less than about 70 mg) contained somewhat higher concentrations of all free amino acids than larger samples (> 70 mg). This variability was regarded as a major reason for the high SD values. Subjective differentiation between high and low secretors resulted in two slightly different mean ± SD values for HPS free amino acids. However, because it was impossible to determine secretion rates accurately, these means were not further considered; the amino acid levels shown were found to describe adequately the nature of the average free amino acid pool of HPS. It is possible that the individual variations in the amino acid levels could be attributable to the presence of peptidolytic enzymes in HPS. The total free amino acid concentration (in nmol/ml) of HPS was 1200 ± 950 (mean ± SD).

The free amino acid pool of HPS differed from that of the whole saliva (Mäkinen, Lönnberg and Scheinin, 1975) and serum (Mäkinen *et al.*, 1982). The dominant free amino acids in whole saliva of subjects similar to those studied here are, in approximate decreasing order of concentration, glycine, proline, histidine, phosphoserine, alanine and tyrosine. In serum, the dominant free acids are normally alanine, glycine, valine, proline, lysine, serine and leucine, also in a rough decreasing order of concentration. In HPS, the corresponding free amino acids were clearly serine, glutamic acid, alanine, valine and glycine. The almost total absence of free arginine and cysteine (and most likely methionine), as well as the low levels of free proline and tryptophan, were considered typical characteristics of the HPS of our subjects.

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