A survey of salivary kallikrein and amylase in a population of schoolgirls, throughout the menstrual cycle

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Summary

1. The values for kallikrein, amylase and protein were determined in samples of saliva obtained from 220 girls aged 14–18 years.
2. The concentrations of protein and amylase and kallikrein activities (per ml of saliva) were considerably more variable in samples taken in the morning than those in the afternoon.
3. The median amylase activity was about two and a half times greater in the morning than that in the afternoon. No such differences were seen in the median values for protein or kallikrein.
4. Examination of the values for salivary kallikrein during the menstrual cycle showed that there was significantly greater activity during days 29–32 and 1–4 than during the rest of the cycle. This pattern was most marked in the morning values of kallikrein but not apparent either in the morning or in the afternoon values of protein or amylase.

Key words: amylase, kallikrein, menstrual cycle, saliva.

Introduction

In a previous study Bhoola, Dorey & Jones (1974) found that kallikrein activities in the hamster submandibular gland were lowered by ovariectomy and restored by treatment with oestradiol. Because of this oestrogen dependence, the possibility was considered that activities of human salivary kallikrein may be altered by variations in steroid hormones that occur during the menstrual cycle. The kallikrein, amylase and protein content of saliva of a population of schoolgirls was determined and used to ascertain whether the enzyme values altered during the menstrual cycle.

Methods

Population surveyed

Saliva samples were obtained on 1 July 1975 from 220 girls out of a total of 423 attending a school, between the ages of 14 and 18 years (Fig. 1). The girls not included either were absent or had other commitments.

Collection of saliva samples

Each girl provided two samples of saliva, one in the morning (10.00–11.00 hours) and one in the afternoon (14.00–16.00 hours). The mouth was rinsed with 0.9% NaCl solution, 0.2 ml of 0.5% citric acid was applied to the tongue and mixed saliva collected for 2 min and immediately frozen. The girls were all experienced in collecting saliva in this way. The age, number of days from the start of the previous menstrual period, age of menarche and details of any medications being taken were recorded.

Assay of enzymes and protein

The samples were allocated to three groups, those of every third girl presenting herself at the collection point in the morning being allocated to a
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FIG. 1. Histogram of the age distribution of schoolgirls who provided the saliva samples for the survey.

given group. The samples were assayed over a period of about 6 months, each sample being frozen for removal of aliquots for assay and then immediately refrozen. This treatment did not appear to alter the activity of the enzymes over the assay period. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

Kallikrein (EC 3.4.21.8, formerly EC 3.4.4.21) was assayed by the enzyme ethanol method (Trautschold & Werle, 1961) with benzoyl-L-arginine ethyl ester as substrate. The assay medium (3.0 ml) for measuring kallikrein activity contained 2 mmol of benzoyl-L-arginine ethyl ester, 1 mmol of nicotinamide-adenine nucleotide/l, 0.167 mg of yeast alcohol dehydrogenase (EC 1.1.1.71; Boehringer)/ml and 2.7 ml of buffer solution (150 mmol of semicarbazide hydrochloride/l, 150 mmol of tetrasodium pyrophosphate/l and 22 mmol of glycine/l, pH adjusted to 8.3 with NaOH, 2 mol/l solution). The rate of hydrolysis of benzoyl-L-arginine ethyl ester at 366 nm was followed for 3 min at 25°C in a Pye-Unicam SP. 1800 recording spectrophotometer. The change in absorbance per minute (×10³) was used to express kallikrein activity as units/ml of saliva.

Amylase (EC 3.2.1.1) activity was assayed by a modification of the method of Dahlquist (1962). The dinitrosalicylate reagent was prepared by dissolving 10 g of 3,4-dinitrosalicylic acid, 16 g of NaOH and 300 g of potassium sodium tartrate in deionized distilled water. The starch solution (20 mg/ml) and the maltose standard (10 mg/ml in 5 mmol/l Sorensen's buffer, pH 6.9) were also freshly prepared. For each assay, reagent blank, standard and sample tubes were prepared in duplicate; each tube contained 50 μl of starch solution, 50 μl of buffer and either 50 μl of distilled water or 50 μl of an appropriate dilution of standard or 50 μl of saliva sample. After 3 min incubation at 25°C, 100 μl of dinitrosalicylic acid reagent was added and the tubes were heated for 10 min at 100°C to allow development of the chromophore. Cold distilled water (3 ml/tube) was then added and the tubes were placed on ice for 30 min. The standards and samples were read against the reagent blank in a Pye-Unicam SP. 500 at 530 nm and amylase activity was expressed as units/ml of saliva (1 unit = 1 mg of maltose formed/min).

Enzyme values relative to saliva volume were used for the statistical analysis. Values relative to protein were not used because expressing them in this way did not appreciably reduce the coefficients of variation; on the contrary the coefficient of variation for the morning kallikrein values was more than doubled.

Statistical analysis of data

Data from six girls taking contraceptive pills were not included in the analyses, whereas results from 15 girls who were taking other medications, mostly for hayfever, were included. Samples for which incomplete data were available were excluded. Nonparametric statistical methods (Colquhoun, 1971) have been used for comparison as the data (Fig. 2) were not distributed either normally (testing Goodness of Fit with χ², all P < 0.001 with 7 d.f.) or lognormally (all P still < 0.05). The differences between morning and afternoon specimens of the same individual were also not distributed normally (all P < 0.01).

Results

Results of the kallikrein, amylase and protein determinations on the morning and afternoon samples of saliva obtained from the 220 schoolgirls are summarized in Fig. 2. The scatter of the values for protein, amylase and kallikrein in the morning saliva samples is considerably greater than that of those in the afternoon samples (Fig. 2).
Salivary kallikrein and the menstrual cycle

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**FIG. 2.** Frequency distributions of saliva content of (a) protein, (b) amylase and (c) kallikrein in morning saliva samples (■) and afternoon samples (□). The data were allocated to groups whose width was that of half the standard deviation. Arrows indicate the mean values and □ indicates the median values.

The variation in protein and kallikrein do not reflect differences between the girls because there was no correlation between the morning and afternoon values from the same girl (Spearman’s Rank correlation coefficients negative, with $P < 0.05$). There was some correlation between morning and afternoon amylase activities from the same girl, but examination of the correlation diagram showed that little of the total variation could be attributed to differences between individuals. Despite the large differences in scatter between the morning and afternoon results, there is little difference between the median values of kallikrein or protein (Table 1 and Fig. 3). Even though the difference in kallikrein

**FIG. 3.** Time course of changes in (a) protein, (b) amylase and (c) kallikrein in morning and afternoon saliva samples. The median values are given, together with their confidence limits as given by the ranked values corresponding most nearly to the 70% probability value. The numbers below the limits indicate the number of values in that group. Where there is an even number of values the median is represented by the mean of the two middle values.
TABLE 1. Comparison of enzyme activities and protein concentrations in saliva samples

The confidence limits are given by the values corresponding in rank most nearly to the 70% probability level. The median of 208 values is given by the mean of the 104th and 105th ranked values. The median of 213 values is given by the 107th ranked value. \( n \) = number of samples for which both morning and afternoon values were available.

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<thead>
<tr>
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<th>Morning</th>
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<th>Afternoon</th>
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<td></td>
<td>Median</td>
<td>Confidence limits</td>
<td>Median</td>
<td>Confidence limits</td>
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<td>Protein (mg/ml)</td>
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<td>Amylase (units/ml)</td>
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<td>9.9</td>
<td>9.6/10.0</td>
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<tr>
<td>Kallikrein (units/ml)</td>
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<td>102/111</td>
<td>94</td>
<td>92/96</td>
</tr>
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values is statistically significant (0.01 < \( P < 0.02 \), median test) it is possibly too small to be physiologically important. In contrast, there was appreciably less amylase activity in the afternoon than in the morning saliva \( (P < 0.001, \) median test; Fig. 3).

The values for kallikrein, amylase and protein during the menstrual cycle are shown in Fig. 3. There is no marked change in the salivary kallikrein activities at mid-cycle when serum oestrogens are known to rise rapidly, and therefore any influence of oestrogens on kallikrein activities can be ruled out. However, the salivary kallikrein activity increases sharply around day 28, the median values during days 29–32 and 1–4 being appreciably higher than those between 5 and 28 days. This perimenstrual peak is most marked in the morning values.

**Discussion**

The survey shows that amylase activity in the schoolgirl's saliva is about two and half times higher in the morning than in the afternoon. This could reflect accumulation of amylase overnight when the girls were not eating or increased rate of formation and secretion in the morning. Either way it may cause differences in digestive capacity during the day and this may have nutritional relevance.

In this survey we observed a perimenstrual peak of salivary kallikrein. We consider this kallikrein peak to be statistically significant, in the sense that it is unlikely to have arisen by chance since it occurs both in the morning and afternoon samples (but more markedly in the morning values) and is not apparent in the results for amylase or protein. Furthermore, the perimenstrual values (days 1–4, 28–32) are significantly increased when compared with the mid-menstrual values (days 4–28) and with those from girls without regular cycles (>32 days) by Kruskal–Wallis test, 0.01 < \( P < 0.005 \). However, caution is necessary in assessing the importance of this finding because it was difficult to provide adequate control for the subjects of our survey and because little correlation was found between morning and afternoon values from the same individual. A large proportion of the variability, particularly in the morning sample, may reflect some uncontrolled factor. It will therefore be necessary to make a series of determinations in each individual throughout a menstrual cycle under controlled conditions before definite conclusions can be drawn.

Although the original concept that human salivary kallikrein may show oestrogen dependence may be ruled out there are a number of factors that might have produced the perimenstrual peak in salivary kallikrein activities. For instance, the increased kallikrein activities may relate to behavioural changes such as altered diet. Another possibility is that the sharp perimenstrual rise in circulating progesterone might increase the synthesis and secretion of kallikrein. In addition, the location of kallikrein in the duct cells of submandibular glands (Brandtzaeg, Gautvik, Nustad & Pierce, 1976; Hojima, Maranda, Moriwaki & Schachter, 1977; Bhoola, Lemon & Matthews, 1977) and in the tubule cells of the kidney (Nustad, Vaaje & Pierce, 1975) suggest that this enzyme may be involved in the control of electrolytes (Marin-Grez, Cottone & Carretero, 1972; Mills & Ward, 1974). Patients with primary aldosteronism also seem to secrete higher amounts of kallikrein in urine (Margolius, Geller, Pisano & Sjoerdsman, 1971). The perimenstrual peak of kallikrein might therefore reflect the changes in water and electrolytes that are known to occur during this phase of the cycle.
Acknowledgments
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References