Arch Otorhinolaryngol (1986) 243:138–140

Archives of
Oto-Rhino-Laryngology

Influence of sex and age on kallikrein excretion in stimulated human parotid saliva

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Summary. We determined the activity of parotid salivary kallikrein in 65 healthy males and females. We then formed two groups of probands by age. The mean age in the first group ($n=35$) was 24.5 ± 1.7 years, whereas the mean age in the second group ($n=30$) was 58.1 ± 10.5 years. The activity of the glandular kallikrein was measured with a chromogenic substrate. The salivary kallikrein excretion in the younger females was higher when compared with that in the younger males. No sex differences concerning salivary kallikrein activity could be observed in the second group. A possible influence of female sex hormone on salivary kallikrein excretion is discussed.

Key words: Kallikrein – Saliva – Sex

Introduction

Salivary glands are known to contain kallikrein, which is a serine protease [9]. Regulation of its synthesis and physiological function in the parotid gland are thus far not completely understood. Its localization in the apical part of striated duct cells indicates that it has an excretory character [5], while Ørstavik et al. [8] have suggested that the kallikrein-kinin system plays a role in the regulation of glandular blood flow.

Several investigations have been performed on the secretory pattern of glandular kallikrein in parotid saliva [3, 10]. Most of these studies used the BAEE-esterase assay for the measurement of kallikrein activity [11]. A more sensitive and specific method for the determination of glandular kallikrein was introduced by Amundsen et al. [1] in 1979. Using this latter method, we studied the flow-rate dependent excretion of glandular kallikrein in human parotid saliva of healthy probands and analyzed it in relation to sex and age.

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Material and methods

Parotid saliva was collected from 65 healthy male and female volunteers. The probands were divided into two age groups: the mean age was 24.5 ± 1.7 years in the younger group ($n=35$) and 58.1 ± 10.5 years in the older group ($n=30$).

To exclude circadian and dietary influences on kallikrein excretion, all investigations were performed between 6.30 and 8.00 a.m. after volunteers were fasted overnight. In order to sample isolated parotid saliva, a polyethylene catheter was introduced into Stensen's duct. Salivary flow was stimulated gustatorily by oral application of 5% citric acid. Immediately after sampling, the glandular kallikrein activity was measured by use of an amidolytic essay: salivary kallikrein splits the substrate H-D-Val-Leu-Arg-pNA (S2266 Kabi, Stockholm). The rate of *p*-nitroanilin (pNA) formation was then determinated photometrically at 405 nm. The results obtained from these studies are presented as mean values ± standard deviation. The Mann-Whitney-Wilcoxon test was used for analysis of statistical significance.

Results

In the older group ($n=30$), the mean value of the salivary flow rate was 0.79 ± 0.31 ml/min. The kallikrein activity in this collective ranged from 0.1 to 8.94 U/l, and averaged 4.94 ± 2.7 U/l. The analysis of regression showed no correlation between salivary flow rate and kallikrein activity in the older probands ($r=+0.06$).

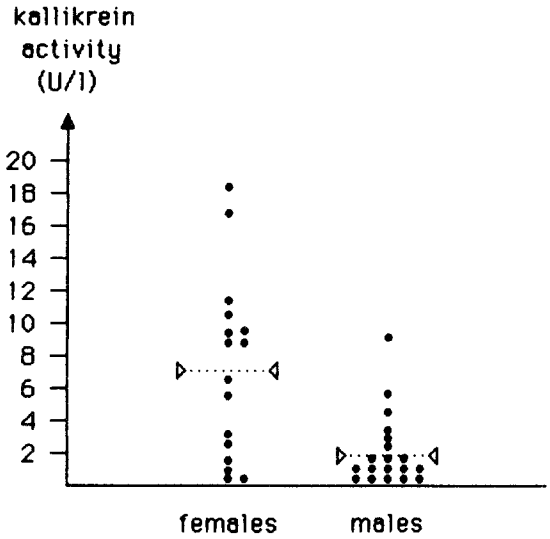

Fig. 1. Kallikrein activity in the parotid saliva of young women and men (▷------◁ = mean value). Mean age, 24.5 ± 1.7 years

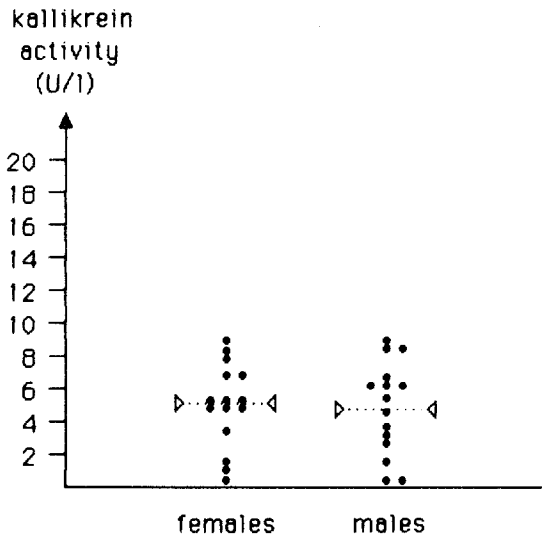

Fig. 2. Kallikrein activity in the parotid saliva of older women and men (▷------◁ = mean value). Mean age, 58.1 ± 10.5 years

In the younger group ($n = 35$), the mean value of the salivary flow-rate was 0.62 ± 0.43 ml/min. The kallikrein activity ranged from 0.1 to 18.3 U/l, and averaged 4.13 ± 4.8 U/l. The analysis of regression showed a weak negative correlation between the salivary flow rate and the kallikrein activity in the younger subjects ($r = -0.34$). There was also no significant influence of contraceptive hormones on kallikrein excretion or on salivary flow-rate in the younger women tested. In the older female probands ($n = 15$), the mean value of the salivary flow-rate was 0.88 ± 0.39 ml/min and the mean value of the kallikrein activity was 5.05 ± 2.6 U/l. The salivary flow rate in the older male subjects ($n = 15$) averaged 0.7 ± 0.33 ml/min and the mean value of the kallikrein activity was 4.8 ± 2.8 U/l (Fig. 2).

In the younger female probands ($n = 16$), the mean value of the salivary flow-rate was 0.42 ± 0.26 ml/min and the mean value of the kallikrein activity was 7.1 ± 5.6 U/l. The salivary flow-rates in the younger male subjects ($n = 19$) averaged 0.79 ± 0.47 ml/min, while the mean value of the kallikrein activity was 1.9 ± 2.4 U/l (Fig. 1).

Discussion

Investigations by Maier et al. [7] have shown that the excretion of salivary kallikrein can be used as an indicator of destructive processes in the duct system of the human parotid gland, and thus may be of diagnostic value. In order to evaluate salivary kallikrein activity in patients suffering from different salivary gland diseases, a basic knowledge of the influence of sex and age on the excretion of the enzyme into parotid saliva is necessary to establish normal values.

Our present study has shown no significant influence of age on the flow-rate or kallikrein activity in human parotid saliva. Heidland et al. [4] found a weak negative correlation between salivary flow-rate and kallikrein activity in younger subjects. In contrast to this, there was no correlation between flow rate and kallikrein activity in older subjects. We also compared results in males and females, and we found definite sex differences in the younger collective: the female probands had a significantly lower salivary flow-rate ($P < 0.004$) and a significantly higher activity of salivary kallikrein ($P < 0.002$). In contrast, there were no differences in flow rate and kallikrein activity in the older male and female subjects.

Since there was no significant correlation between salivary kallikrein concentration and flow-rate, the elevated kallikrein excretion in the parotid saliva of young females can not be related to the reduced salivary flow rate in these subjects.

It is our belief that the enhanced salivary excretion of this enzyme is caused by the female sex hormones. The influence of estrogens on the function of human salivary glands has been discussed in a previous study [6]. Bhoola et al. [2] described an estrogen-induced synthesis of kallikrein in hamster submandibular glands. In a clinical study of 14- to 18-year-old grils, Bhoola et al. also found alterations of kallikrein activity in mixed saliva during the course of each girl's menstrual cycle.

Our finding of an absent sex difference in salivary flow-rate and kallikrein activity in parotid saliva in older probands suggests an influence of menopausal alterations on sex hormone levels.

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Received October 8, 1985 / Accepted October 27, 1985