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Studies on human salivary kallikrein

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KALLIKREINS from human whole saliva have been partially purified and characterized by Moriya *et al.*[1] A study on the kallikrein activity in human whole saliva from normal subjects and those with periodontal disease has been recorded by Sallay and Nador.[2] Despite the potency of the kallikrein activity in human saliva, there are few data as to the origin of the activity in whole saliva (i.e. what are the individual contributions of the parotid, submaxillary and sublingual secretions?). In animal studies activity has been reported as occurring mostly in the submaxillary gland.[3] In the current study we have determined the relative activity of kallikrein in human parotid, submaxillary and whole saliva. Since hypertensive patients as a group show lower rates of salivary flow as well as lower salivary sodium concentration when compared with normal subjects,[4] we have also compared the kallikrein activity in the secretions of hypertensive patients with the activity of normal subjects.

METHODS

Parotid saliva was collected by utilizing a modified Lashly cup.[5] Submaxillary saliva was collected with the Block–Brotman collector.[6] Both parotid and submaxillary saliva were obtained by reflex stimulation (2% citric acid swabbed on tongue). Whole saliva was paraffin stimulated and centrifuged to remove any particulate matter before use. Since the composition of some salivary constituents is affected by variations in the rate of flow of saliva, the kallikrein activity of samples collected at different rates of flow was determined. The different rates of flow were elicited by a graded response technique[4] (1%, 2%, 3% citric acid swabbed on tongue).

Kallikrein activity was determined both by its esterase activity on benzoyl L-arginine ethyl ester (BAEE) and by its ability to form kinin from dog plasma substrate.

Esterase activity. One ml BAEE, 0·0005 M, in 0·01 M Tris buffer at pH 8·5 was used as substrate for 0·25 ml saliva (parotid, submaxillary or whole). The substrate was incubated at 37° for 5 min before the addition of the saliva. The esterase activity was followed by the increase in optical density at 253 mm with time.[7, 8]

Bioassay. Heated dog plasma, prepared by the method of Horton,[9] was used as substrate for the bioassay of kallikrein activity. Saliva (0·1 ml) was incubated with an equal amount of substrate and 0·4 ml Tris buffer, pH 8·0, for 10 min at 37°. Kinin produced by kallikreins was then assayed by the contraction of the guinea pig ileum. Control samples of the plasma or the saliva alone did not produce measurable activity.

Sample handling. Activity was measured in freshly collected, refrigerated and frozen samples. A comparison was also made of samples collected in glass and plastic vessels. Trasylol (Bayer Corp.) was used to study the inhibition of kallikrein activity.

Hypertensive subjects. The hypertensive saliva samples were collected from ambulatory patients under treatment in the Nephritis-hypertension Clinic, Columbia-Presbyterian Medical Center. Approximately $\frac{2}{3}$ of these patients were receiving medication for hypertension. These samples were frozen for 2–6 months before assay. The control samples were obtained from normotensive subjects and were frozen for about the same length of time before use.

RESULTS

Comparative activities. Table 1 shows the kallikrein activity (BAEE activity) of the whole saliva and the separately collected secretions. As may be seen, the whole saliva activity was much greater (2–3 times) than the activity of the separately collected secretions in half of the cases studied. The rest of the subjects produced whole saliva whose kallikrein activity was equal to or slightly greater than either the parotid or submaxillary secretion activity alone. In one case the comparative activities established by utilizing the BAEE system were checked by bioassay. The kallikrein activity of the whole saliva was greater than the parotid activity for this subject when determined either by bioassay

TABLE 1. COMPARATIVE BAEE ACTIVITY OF FRESHLY COLLECTED SALIVARY FLUIDS*

Subject	Parotid	Submaxillary	Whole saliva
1	45	22	103
2	66	108	95
3	36	36	104
4	21	26	27
5	18	13	25
6	30	55	76
7	21	41	33
8	58	53	83
9	91	47	57
10	76	49	174
11	22	11	
12	20	45	

* A unit is defined as that amount of enzyme causing a change at 253 μ in 0·001 O.D. units/min/0·1 ml saliva added to the cuvette containing 0·0005 M BAEE, 0·05 M Tris, pH 8·5, in a total volume of 2·5 ml at 37°.

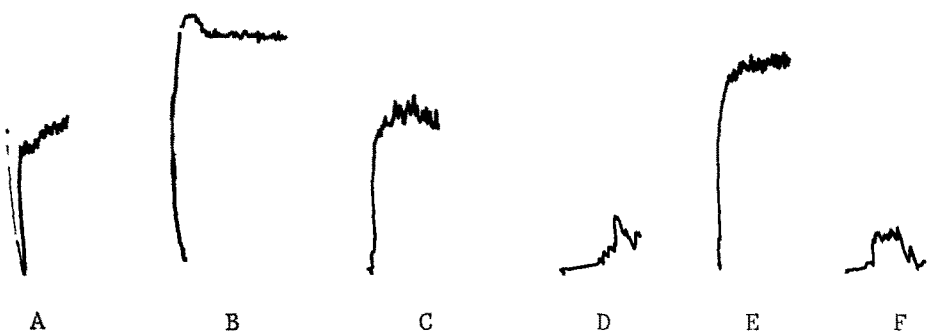

FIG. 1. Assay of salivary kallikrein on the guinea pig ileum. A, 10 ng bradykinin; B, 20 ng bradykinin; C, parotid saliva + dog plasma; D, parotid saliva + dog plasma + 50 KIU trasylol; E, whole saliva + dog plasma; F, whole saliva + dog plasma + 50 KIU trasylol. BAEE activity of the parotid and whole saliva are indicated in Table 1 (subject 5). Details of the bioassay are in the text.

or esterase activity (Fig. 1). Figure 1 also indicates that trasylol inhibits this bioassay. Trasylol inhibited the BAEE systems as well, indicating the kallikrein nature of the esterase.

Since in half of the cases the whole saliva exhibited much greater activity than either of the separately collected secretions, we decided to investigate the possible presence of an activator substance in this secretion. Whole saliva was boiled for 10 min to destroy its kallikrein activity. In ten instances, when 0·1 ml of boiled whole saliva (no endogenous activity) was added to parotid secretion, kallikrein activity was greater than the activity of the parotid secretion alone. In only three instances was there no difference in activity between parotid secretion alone and parotid secretion plus the boiled whole saliva. Increased activity after the addition of boiled whole saliva to parotid saliva was also demonstrated by bioassay.

There were no differences observed in the kallikrein activity of the samples collected at different rates of flow elicited by the graded response technique.

Sample handling. There was no significant difference between those samples collected in plastic and those collected in glass. Fresh and frozen samples had comparable activity; refrigerated samples had reduced activity.

Hypertensive subjects. Kallikrein activity in parotid saliva of hypertensive subjects was not significantly different from the activity found in control subjects (Table 2).

TABLE 2. PAROTID KALLIKREIN ACTIVITY*

Subjects	
Hypertensive	Control
Mean 42	54
Range 21–87	27–90

* Values are for twelve pairs of subjects: see units, Table 1.

DISCUSSION

For all practical purposes there seems to be little or no difference in the humans studied between the kallikrein activity of parotid and submaxillary secretions. This is in contrast to earlier findings in animals,[3] which suggested that the submaxillary gland was the major source of whole saliva kallikrein The whole saliva often shows a much higher level of kallikrein activity. The presence of a possible "activator" for kallikrein activity in the whole saliva of some patients has been noted. The fact that some subjects exhibit this phenomenon and others do not is of interest. The nature of this activator remains to be established.

Trasylol inhibited the activity of the secretion completely in the chemical system and markedly reduced it in the biological. This suggests strongly that the enzyme is kallikrein. The low level of activity remaining in the biological system may be a result of the sensitivity of the muscle or may suggest the presence of a second enzyme which is not sensitive to trasylol but which still can form kinins from the plasma substrates.

The twelve pairs of samples of parotid secretions from hypertensive and control subjects showed no significant differences in kallikrein activity. Since the parotid saliva from these same hypertensive individuals shows reduced rates of flow and sodium concentration when compared with control subjects,[4] it would appear that the kallikrein activity in the secretion is not a factor in this change in the parotid secretion. This would tend to support the work of Bhoola *et al.*[10] who feel that kallikrein activity and subsequent vasodilatation are not responsible for the work of the salivary glands, as postulated by Hilton and Lewis.[11] It is not known, however, if the concentration of kallikrein in the salivary secretion reflects activity levels in the gland itself. The whole saliva was not used in these measurements because of the variable presence of an activator which affected kallikrein activity in some individuals as well as the presence of numbers of leukocytes[12, 13] and bacteria in this secretion.

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