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AMINOPEPTIDASE B IN HUMAN GINGIVAL EXUDATE AS AFFECTED BY REDUCED ORAL HYGIENE AND SUGAR DIET

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The activity of aminopeptidase B, an arginine aminopeptidase probably involved in the inflammatory process, was measured in human gingival exudates collected from persons kept on various sugar diets. The test persons were divided into four groups, receiving in their diet either sucrose, glucose, fructose, or xylitol during a four days period, after which the gingival index and the activity of aminopeptidase B in the gingival exudates were determined. The same measurements were carried out after a two week period of normal diet and good oral hygiene. The activity of aminopeptidase B was high in all samples after the four days sugar diet, increasing in the following order: glucose, fructose, sucrose, xylitol. After the two week normal period the activity of aminopeptidase B was reduced to one half or one third of the initial values.

Inflammation has long been postulated as the most important mechanism leading to the destruction of the periodontium. The question of the initiation of inflammation reaction is still open for investigation. The role of aminopeptidase B in gingivitis in this sense has been discussed earlier in connection with the occurrence of this enzyme in the periodontium, especially in the basal cell layer of the epithelium (*Paunio & Mäkinen,* 1970). Aminopeptidase B is a chloride activated arginine aminopeptidase which also acts on lysyl peptides (*Hopsu, Mäkinen & Glenner,* 1966 a, 1966 b). Its occurrence in oral tissues other than periodontal tissues has been demonstrated earlier (*Mäkinen, Brummer & Scheinin,* 1970). Aminopeptidase B is considered to be an enzyme partially responsible for the formation of bradykinin, an inflammatory mediator (*Hopsu-Havu, Mäkinen & Glenner,* 1966 c),

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although some other functions for the enzyme have also been suggested (*Mäkinen*, 1969).

The flow of gingival exudate is markedly increased in gingivitis (*Brill & Björn*, 1959), and is a sign of inflammation in the periodontal tissues. In this current study the attempt was to determine aminopeptidase B activity in gingival exudate from an irritated gingiva. The irritation was caused in test persons by a special sugar diet combined with reduced oral hygiene.

The gingival exudate has so far been subject of a few enzyme studies only. Both oxidative and hydrolytic enzymes have been earlier observed in gingival exudate (*Körber*, 1967). *Winer et al.* (1970) showed that different enzyme activities occurred in the gingival exudate and that β-D-galactosidase activity increased in the crevicular fluid collected from affected sulcusis.

MATERIALS AND METHODS

The chemicals used in the experiments were supplied by E. Merck AG (Darmstadt, W. Germany) unless otherwise stated.

37 dental students were used in the study and divided into four groups according to their diet. The basic diet was normal in all the groups, varying only in the type of carbohydrate (xylitol, glucose, fructose and sucrose) present in the diet as described elsewhere (*Scheinin & Mäkinen*, 1971; *Mäkinen & Scheinin*, 1971). During the test period of 4 days no oral hygiene was allowed. The test persons were examined both after the test period and after 14 days normal oral hygiene and normal diet (control period). As to details concerning test arrangements, see *Scheinin & Mäkinen* (1971).

The gingival exudate was collected with filter paper strips at the opening of the most markedly affected sulcuses according to *Löe* and *Holm-Pedersen* (1963) — 2 strips per individual and 1 strip per sulcus. The gingival condition was scored according to *Löe* and *Silness* (1963). The strips were immediately immersed in test tubes in 0.6 ml of 0.05 M phosphate buffer (pH 7.2) for 30 min at +4°C. The test tubes were shaken every 5 min by hand. The resulting solutions were used as enzyme preparations. The activity of aminopeptidase B was determined in mixtures composed of 0.3 ml of 0.05 M phosphate buffer (pH 7.2), 0.1 ml of enzyme preparation and 0.1 ml of substrate (N-L-arginyl-2-naphthylamine; Mann Research Laboratories, Inc., New York, N.Y., USA) as described elsewhere in more detail (*Mäkinen*, 1968). The ability of the gingival exudate to hydrolyze this substrate was demonstrated in the presence and absence of 0.2 M NaCl, which produces the maximal rate of the hydrolysis of N-L-arginyl-2-naphthylamine catalyzed

by aminopeptidase B (*Mäkinen*, 1969; *Mäkinen & Mäkinen*, 1971). Although the physiological concentration of sodium chloride is about 0.154 M, it was advantageous to assay aminopeptidase B in 0.2 M salt concentration. The protein content of the samples was measured by the Folin-Ciocalteu method (*Layne*, 1965).

RESULTS

Fig. 1 and Table I show the results. In all four sugar groups, the activity of aminopeptidase B in the gingival exudate was at least twice as high after the test period as that observed after two weeks normal oral hygiene and normal diet. The lowest rates of the hydrolysis were observed in samples representing the fructose group both in the test and control samples. The highest rates of the hydrolysis were measured in samples representing the xylitol group. The control values behaved in a similar way. The enzyme activities were also high in the exudate representing the sucrose group. 0.2 M NaCl increased the rate of the hydrolysis in all samples (Fig. 1). In only one case the enzyme activity was lower (absorbance = 0.272) after the test period than after two weeks normal diet and normal oral hygiene (absorbance = 0.285).

The gingival tissue was affected in all test persons by the carbohydrate diet and bad oral hygiene, regardless of which sugar was consumed (Table I). The gingival index, which was generally high after the test period, decreased markedly during the two weeks control period. The protein content was also increased in the gingival exudate during the test period (Table I), except for the fructose group. The protein concentrations were not used to calculate specific activities because in the present approach the main purpose was only to determine the activity of aminopeptidase B in gingival exudate. Consequently, no attention was paid to the ultimate reason for the differences observed, whether due to the increase of proteins or the activity of the enzyme studied.

DISCUSSION

Aminopeptidase B hydrolyses only *N*-L-arginyl- and *N*-L-lysyl-2-naphthylamines of a great number of different *N*-L-aminoacyl-2-naphtylamines investigated. These reactions are characterized by an interesting property which has also been the main basis for all suggestions about the physiological role of aminopeptidase B. This is the potent activating effect of chloride ions

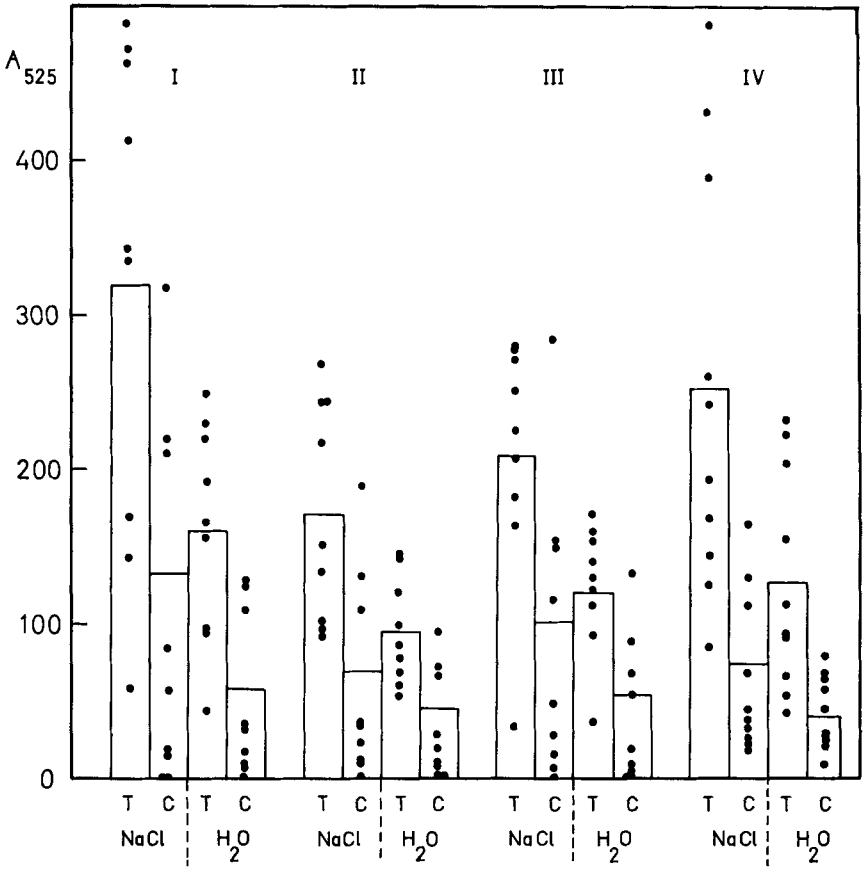

Fig. 1. Activity of aminopeptidase B in gingival exudate after a 4 days period of sugar diet (T) and a 14 days control period (C). The ability of the gingival exudate to hydrolyze *N*-L-arginyl-2-naphthylamine was demonstrated in the presence and absence (water instead) of 0.2 M sodium chloride. I = xylitol group, II = glucose group, III = fructose group, IV = sucrose group. The bars represent the mean values in each group.

of physiological concentration (0.9 % or 0.154 M). The effect of chloride ions in reactions catalyzed by aminopeptidase B has been considered earlier (*Hopsu, Mäkinen & Glenner,* 1966 b, 1966 c; *Mäkinen & Hopsu-Havu,* 1967 a; *Mäkinen,* 1969; *Mäkinen & Mäkinen,* 1971). Although anionic activation of enzymes has been considered uncommon or unspecific, an increasing number of enzymes has been shown to be activated in this way and, at least in the case of aminopeptidase B, the activation has been considered specific (*Mäkinen & Mäkinen,* 1971). The biochemical (*Mäkinen & Paunio,* 1970; *Mäkinen, Brummer & Scheinin,* 1970) and histochemical (*Pau-*

Table I.

The relationship between sugar diet and the activity of aminopeptidase B and protein content of the gingival exudate. The enzyme activity is expressed as absorbance ($\times 10^3$) of the reaction mixtures and the protein concentration as absorbance ($\times 10^3$) of the solutions obtained by extracting the paper strips in 0.6 ml of 0.05 M phosphate buffer, pH 7.2. Because of the unspecificity of the method used in the determination of proteins, only the absorbances are shown

Sugar group	Enzyme activities			Proteins			GI		
	T	C	D	T	C	D	T	C	D
Xylitol	321.0	132.0	189.0	105.3	24.4	80.9	0.72	0.32	0.40
	(161.0)	(100.0)		(45.3)	(19.8)		(0.18)	(0.14)	
Glucose	172.2	68.5	103.7	50.2	27.2	23.0	0.61	0.26	0.35
	(66.0)	(61.5)		(25.5)	(13.4)		(0.19)	(0.17)	
Fructose	209.1	100.8	108.3	48.9	60.8	—11.9	0.64	0.12	0.52
	(76.8)	(89.1)		(19.5)	(13.8)		(0.06)	(0.08)	
Sucrose	252.4	74.3	178.1	78.9	34.9	44.0	0.81	0.27	0.54
	(130.0)	(54.5)		(48.2)	(21.3)		(0.17)	(0.10)	

T = situation after the test period when the test persons were on a four days sugar diet;
C = control period, *i.e.* the values shown were obtained after a two week period of good oral hygiene;
GI = gingival index;
D = difference between T and C
The value in the parenthesis = standard deviation

nio & Mäkinen, 1970; *Mäkinen & Paunio,* 1971) demonstration of aminopeptidase B in crude preparations of various oral tissues has been mainly based on the suggested specificity of the activation of aminopeptidase B by chloride ions. This is also implied in the present study, where the rate of the hydrolysis of *N*-L-arginyl-2-naphthylamine catalyzed by gingival exudate was potently increased by the addition of 0.2 M NaCl. Crude enzyme preparations like those used in the present study contain certain amounts of sodium chloride which, however, are too low to cause maximum activity of aminopeptidase B. They also contain enzymes other than aminopeptidase B which are capable of hydrolyzing *N*-L-arginyl-2-naphthylamine, but all experiments so far carried out have shown that only the hydrolysis catalyzed by aminopeptidase B requires about 0.15 M NaCl for maximum activity. The assumption about the specificity of the activation of aminopeptidase B by chloride ions seems to be valid on the basis of experiments carried out in this laboratory on a large number of enzymes hydrolyzing various *N*-L-aminoacyl-2-naphthylamines.

The results of the present study suggest that aminopeptidase B is present in the gingival exudate, because the rate of the hydrolysis of *N*-L-arginyl-2-naphthylamine was indeed increased in reaction mixtures containing 0.2 M NaCl. The fact that the NaCl-concentration in the intercellular space resembles that concentration which is shown to be the optimum for the activity of aminopeptidase B, suggests that this enzyme becomes active when it is liberated outside the cells. Therefore, it could be thought that the increasing activity of aminopeptidase B in the gingival exudate is a sign of increased damage of the epithelial cells in the gingival fluid and/or of damage on the connective tissue cells. The present stage of aminopeptidase B research indicates that the enzyme does not occur in leucocytes.

It has been suggested that aminopeptidase B is capable of converting kallidin-10 (a decapeptide) to bradykinin (a nonapeptide) which is considered to be an inflammatory mediator (*Hopsu, Mäkinen & Glenner,* 1966 c). On the other hand, there are biochemical data (*Mäkinen*, to be published) suggesting that aminopeptidase B would be able to decompose bradykinin further to smaller peptides and even amino acids. Consequently it could be suggested that aminopeptidase B displays either or both of the following two roles in the tissues:

1. Converting kallidin-10 to bradykinin
2. Converting bradykinin to inactive breakdown products.

Tonzetich et al. (1969) observed a correlation between the rate of bradykinin inactivation by saliva and the periodontal status. The inactivation process proceeded at a more rapid rate in the group with definite signs of periodontal disease than in a »normal» group. These results could probably be explained by the presence of aminopeptidase B in the gingival exudate, from where it is transferred elsewhere in the oral cavity.

The results in the present study showed that the difference of aminopeptidase B activity in the gingival exudate between the test and control situation was high in the xylitol- and sucrose groups. The differences observed did not necessarily derive from the specific effect of xylitol and sucrose on the gingiva when compared to the effect of glucose and fructose. The products which the test persons had to consume during the diet period, made from xylitol were much more ragged and harder than those made from sucrose, glucose and fructose. It is thus possible that the differences obtained were a consequence of different mechanical irritations.

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