The content of arginine aminopeptidases, hexosamine, and uronic acid sugars in gingival exudate as affected by short term sugar diets

KEIJO U. PAUNIO, KAUKO K. MÄKINEN & ARJE SCHEININ

Institute of Dentistry, University of Turku, Finland

Paunio, K. U., Mäkinen, K. K. & Scheinin, The content of arginine aminopeptidases, hexosamine, and uronic acid sugars in gingival exudate as affected by short term sugar diets. *Acta Odont. Scand.* 31, 193—199, 1973.

The specific activity of arginine aminopeptidases (which are properly represented by aminopeptidase B) and the amount of hexosamines, uronic acids, serotonin and histamine was determined in gingival exudates obtained from persons kept for five days on various mild sugar diets (including sucrose, xylitol, fructose-xylitol, fructose-sorbitol, fructose-glucose, and sucrose-maltose). The sugars consumed during reduced oral hygiene did not differ as regards their ability to induce aminopeptidase B-activity. The enzyme activity towards *N*-L-arginyl-2-naphthylamine was somewhat higher in fructose-sorbitol, glucose-fructose, fructose-xylitol and xylitol groups than in the sucrose and sucrose-maltose groups. The sugars did not lead to any differences in the amount of uronic acids and hexosamines in the exudates. This concerned histamine and serotonin as well.

Key-words: Enzymes; diet; gingival exudate; aminopeptidases; sugars; inflammation

Keijo U. Paunio, Institute of Dentistry, University of Turku, Lemminkäisenk. 2, SF-20520 Turku 52, Finland

The release of biogenic amines (histamine and serotonin) and their effect on the vascular system is a well known early step in the inflammation of the connective tissue. In connective tissue hexosamines are constituents of many polysaccharides, for example, of the acid glycosaminoglycans. *Hara & Löe* (1969) and *Paunio* (1971) showed the presence of such hexosamines in the gingival exudate which was suggested to reflect the connective tissue metabolism and/or the metabolism of the micro-organisms in the oral cavity.

The activity of aminopeptidase B (an arginine aminopeptidase) in gingival exudate was observed to be elevated when provoked by reduced oral hygiene and consumption of certain sugars (*Paunio, Mäkinen & Scheinin*, 1971). Because aminopeptidase B has been suggested to play a role in the inflammation process (*Hopsu, Mäkinen & Glenner*, 1966) its activity and presence in the oral cavity should be considered. The present investigation was carried out in order to elucidate the possible effect of the consumption of different sugars on the activity of this enzyme (or, in general, arginine aminopeptidases) in gingival exudate. In addition, it was attempted to estimate the

Received for publication, November 25, 1972.

amount of histamine, serotonin, hexosamines and uronic acids in the gingival exudate obtained from persons put on different sugar diets.

MATERIAL AND METHODS

The chemicals used in the experiments were obtained from E. Merck AG (Darmstadt, W. Germany), unless otherwise stated. 61 Dental students were used as test objects. They were divided into 6 groups according to the sugars used as sweetener (sucrose, fructose-sorbitol (1:1), fructose-glucose (1:), fructose-xylitol (1:1), xylitol and sucrose-maltose (9:1). The basic diet was normal in all groups varying only in the type of the sweetener used. The experiments were carried out in three separate steps as shown in Fig. 1.

During the diet periods (Period A and Period B) the test persons 1) washed their mouths with 100 ml sugare solutions/(10% w/v, 5 times a day, 2) sweetened the used coffee, tea, etc. with the particular sugar, 3) and consumed sweet rolls prepared with the aid of the sugars. During Periods A and B the test persons refrained from brushing their teeth. More detailed information concerning the dietary regime and other details have been published earlier (*Scheinin* & *Mäkinen*, 1972).

The gingival exudate was collected with filter paper strips at the opening of different gingival sulci (5+ — +5 and 5— — —5) according to *Löe* and *Holm-Pedersen* (1963) (16 strips per person and

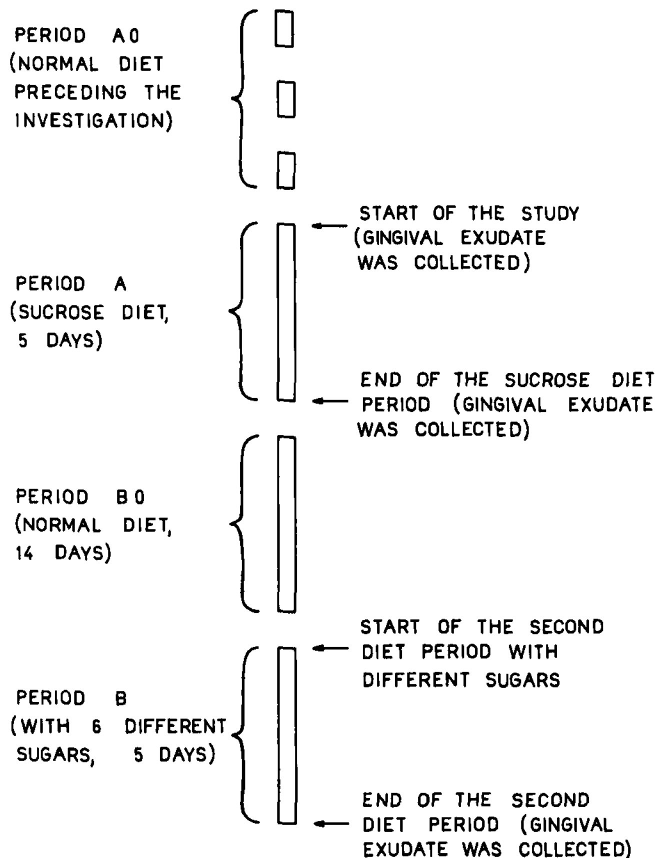

Fig. 1. Overall plan of the study. More details concerning the dietary regime have been described elsewhere (*Scheinin* & *Mäkinen*, 1972).

1 strip per sulcus; collecting time was 2 min per strip). The strips from each person were immediately divided into two pools: 10 strips were used for pool I and 6 strips for pool II. Pool I was stored in cold (+4° C) until further investigation was performed (after about one week). The six strips of pool II were immediately immersed in 1.0 ml of 0.05 M phosphate buffer (pH 7.2) at +4°C. The test tubes were shaken every 5 min by hand during a 30 min period. Eventual turbidity was spun down. The resulting solutions were used as enzyme preparations inexperiments indicated below.

The strips of pool I were treated in the following way. The samples obtained in the beginning and end of period A (Fig. 1) were separately pooled. The samples derived from period B were pooled to form six pools of strips according to the six diet groups. The strip pools were immersed in distilled water (100 strips per 7 ml) for 2 hours at 25°C. The mixtures were gently stirred every 15 min by a glass rod. Eventual turbidity was spun down. The resulting solution was divided into two parts: one for histamine and serotonin determinations and one for hexosamine and uronic acid determinations.

The activity of aminopeptidase B was determined in reaction mixtures composed of 0.3 ml of 0.05 M phosphate buffer (pH 7.2), of 0.1 ml of enzyme preparations (formed by pools II) and of 0.1 ml of substrate (*N*-L-arginyl-2-naphthylamine; Mann Research Laboratories, Inc., New York, N.Y., U.S.A.), as described in more detail earlier (*Mäkinen*, 1968). The ability of the gingival exudate to hydrolyze the substrate mentioned was demonstrated in the presence and absence of 0.2 M NaCl. 0.2 M NaCl leads almost to the maximal rate of the hydrolysis of *N*-L-arginyl-2-napthylamine catalyzed by aminopeptidase B (*Mäkinen & Mäkinen*, 1971).

The protein content of the samples was measured by the Folin-Ciocalteau method (*Layne*, 1965). The hexosamines were estimated by the method of Elson and Morgan (*Blix*, 1948) and uronic acids by the orcinole reaction as described by Dische (1955).

The assay of histamine was based on its coupling to *o*-phtalaldehyde. The fluorescence of the condensation product was measured spectrofluorometrically (*Shore et al.*, 1959). Serotonin was assayed according to the spectrofluorometric method of *Udenfriend et al.* (1955).

RESULTS

Fig. 2 shows the specific arginine aminopeptidase activity of the gingival exudates determined at the end of Period A and B. Prior to the beginning of Period A (at the end of Period AO) the protein concentration of the exudates was too low to permit reliable measurements by the method used. Hence no specific activities for Period AO are given. However, the rates of the hydrolysis catalyzed by arginine aminopeptidases of the samples of Period AO, expressed as M min^{-1}, were generally 1/2 — 1/10 of those measured with enzyme preparations representing Periods A or B. The increase of the protein concentration in the gingival exudates when transfering from Period AO to Periods A and B was due to the reduction of oral hygiene during the latter periods (Fig. 3).

Fig. 2 shows that the specific activity was increased during Period B in all groups. This was most likely due to the lower amount of protein of the exudates during Period B (Fig. 3). It is to be noticed

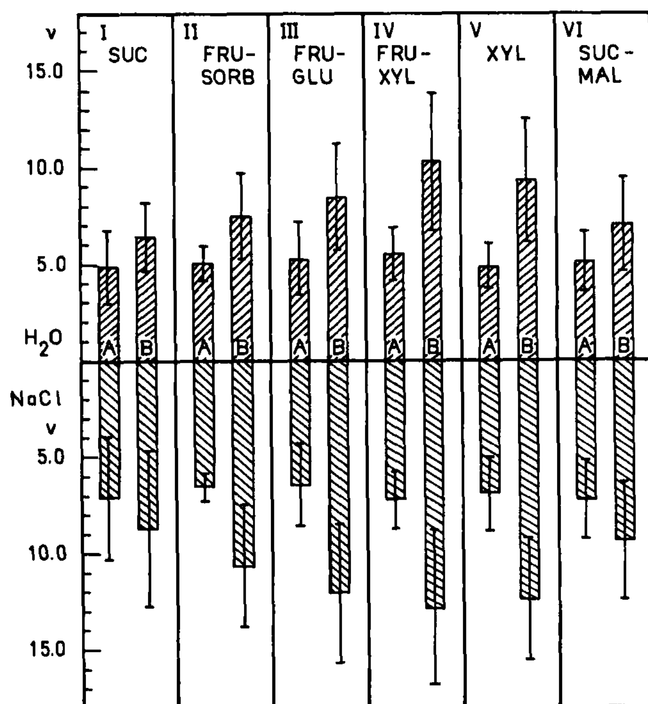

Fig. 2. The specific activity [in liberated μmoles of 2-naphthylamine/(min × mg protein) × 10^3] of enzymes acting on *N*-L-arginyl-2-naphtylamine(=aminopeptidase B-like activity) in the gingival exudate as affected by different mild sugar diets. A = the end of the sucrose diet. B = the end of the particular sugar diet. The rate of hydrolysis in the absence and presence of 0.2 M NaCl, and the standard deviations are indicated.

that in all sugar groups the specific activity at Period A was very similar (columns marked with A in Fig. 2). The addition of NaCl slightly increased the enzyme activity in all sugar groups (lower part of Fig. 2). The rate-enhancing effect of 0.2 M NaCl was about the same in all the sugar groups both after period A and B (Table I).

In sugar groups II, III, IV and V there

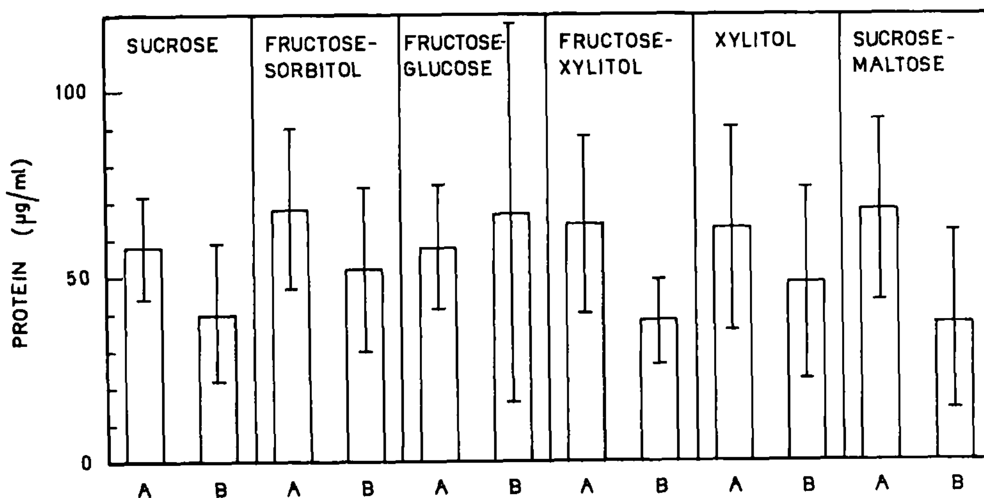

Fig. 3. The content of proteins in gingival exudate as affected by reduced oral hygiene and different sugar diets. A and B have the same meanings and the different diet groups are the same as indicated in Fig. 1. Standard deviations are indicated.

Table I. *The specific activity of arginine aminopeptidases of human gingival fluid (in liberated µmoles of 2-naphthylamine per min and mg protein × 10^3) as affected by different sugar diets. The ratios of the rates obtained in the presence of 0.2 M NaCl and without added salt illustrate the involvement of arginine aminopeptidases (particularly aminopeptidase B-like enzymes) which are rather specifically activated by sodium chloride (0.154 M; the excess when using 0.2 M is deliberate and reduces the unspecific effect of other ions). This ratio was not changed in the groups to any noticeable degree and their mean was the same at both Period A and B*

Sugar group	Specific activity H_2O		Specific activity 0.2 M NaCl		$\nu NaCl/\nu H_2O$
	Period A (all groups on sucrose diet)				
	$\bar{x}$	S.D.$_k$	$\bar{x}$	S.D.$_k$	
Sucrose	4.96	1.92	7.12	3.25	1.43
Fructose-sorbitol	5.16	0.92	6.52	0.74	1.26
Glucose-fructose	5.32	1.92	6.48	2.14	1.22
Fructose-xylitol	5.54	1.39	7.21	1.46	1.30
Xylitol	4.87	1.18	6.90	1.90	1.42
Sucrose-maltose	5.09	1.58	7.21	2.05	1.42
				Mean	1.34
	Period B (all groups on different sugar diet)				
	$\bar{x}$	S.D.$_k$	$\bar{x}$	S.D.$_k$	
Sucrose	6.47	1.78	8.67	4.04	1.34
Fructose-sorbitol	7.56	2.24	10.67	3.25	1.41
Glucose-fructose	8.55	2.82	12.07	3.64	1.41
Fructose-xylitol	10.34	3.57	12.87	4.04	1.24
Xylitol	9.39	3.24	12.43	3.16	1.32
Sucrose-maltose	7.17	2.40	9.41	3.08	1.31
				Mean	1.34

was more chloride-activated enzyme activity towards the substrate used than in groups I or VI, but the ratios mentioned were similar in all groups.

The consumption of different sugars and reduced oral hygiene did not clearly affect the content of hexosamines and uronic acids in the gingival fluid (Fig. 4). Fig. 4 shows the results as means calculated for each Period. This was made even with Period B in spite of the use of the six sugar groups because the values obtained wery similar. However, in the xylitol groups (IV and V) the amount of hexosamines was somewhat lower than in the other groups. The amount of uronic acids was almost identical in all sugar groups.

Only traces of both serotonin and histamine were detectable in the gingival fluid. The amount of these amines was, however, in all sample pools too low to permit any reliable assay.

DISCUSSION

The results of the previous study (*Paunio, Mäkinen & Scheinin*, 1971) showed that the rate of the hydrolysis of *N*-L-arginyl-2-naphthylamine was increased in the gingival exudate due to reduced oral hygiene. It was suggested, basing on the activating effect of sodium chloride, that this enzyme activity would at least partly represent aminopeptidase B.

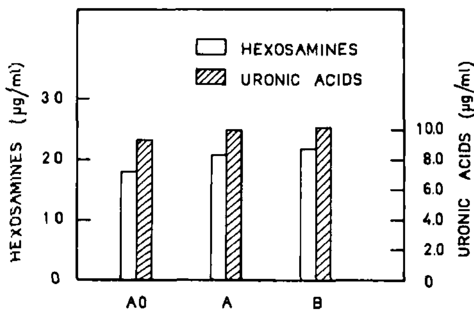

Fig. 4. The content of hexosamines and uronic acids in gingival exudate as affected by reduced oral hygiene and sugar diet. AO, A and B indicate the three experimental periods mentioned in the text and in Fig. 1. Because the gingival fluid of all test persons during Periods AO and A was affected by similar conditions, the paper strips representing these periods were pooled. When the six different sugar pools at Period B were studied, the results were so similar that a mean value was considered sufficient to represent all sugar groups at Period B as well. Hence no separate values for the different sugar groups are given.

One of the purposes of this paper was to elucidate the involvement of enzymes resembling aminopeptidase B, *i.e.* enzymes which are activated by 0.2 M sodium chloride. If the interest is focused to the rate-enhancing effect of 0.2 M NaCl, it is seen that the dietary regime used had not lead to any considerable appearance in the gingival fluid of enzymes resembling aminopeptidase B (Table I). This suggestion is based on the similar ratios of the rates obtained in the presence and absence of 0.2 M NaCl in all sugar groups, as shown in the Table I. If typical aminopeptidase B-like enzymes had been involved, the presence of 0.2 M NaCl would have led to a higher rate of the hydrolysis, revealing any larger amounts of active aminopeptidase B.

Aminopeptidase B may occur in the gingival exudate mainly as a result of gingival inflammation. The present results showed that the enzyme activity towards *N*-L-arginyl-2-naphthylamine was somewhat higher in sugar groups II, III, IV and V than in groups I or VI. It is possible that the different carbohydrates had a different direct or indirect effect on the gingival metabolism in which these enzymes are involved. This explanation is not valid when all the proteins in the gingival exudate are taken into consideration. The protein content was lower in the same ratio after Period B than after Period A in all groups. Group IV was an exception.

It can be claimed that the hexosamines and uronic acids in the gingival fluid were partly of microbial origin as it is known that micro-organisms contain hexosamines and uronic acids. The results in the present study showed, however, that there was no clear increase in the content of hexosamines and uronic acids in the gingival exudate after the test periods. Secondly, the amount of plaque of the test persons was increased from Period AO to Period A in all test groups and from Period BO to Period B in most test groups (*Scheinin & Mäkinen*, 1972). On the basis of these two observations it seems possible that the increased aminopeptidase B-like activity was of gingival and not of microbial origin. So far there is no report on the occurrence in bacteria of an enzyme identical with the aminopeptidase B of mammalian tissues.

The results of the previous study (*Paunio, Mäkinen & Scheinin*, 1971) showed that the difference of aminopeptidase B activity (or more correctly, the activity of arginine aminopeptidases) in the gingival exudate between the test (B) and control periods (A) was higher in xylitol- and sucrose groups than in glucose and fructose groups. It is possible that the differences mentioned in the above paper were a consequence of mechanical irritation caused by the rough consistency of xylitol

toffees used earlier (*Paunio, Mäkinen & Scheinin*, 1971).

However, the present diet did not include any rough material like the xylitol candies used in the first study. Therefore, the somewhat higher specific activities in groups II—V in the present study was most likely due to the specific effect of the particular sugars in promoting the growth of bacteria producing arginine aminopeptidases other than tissue aminopeptidase B. However, it was not the mere rates which were considered important in this study, but rather the rate-enhancing effect of 0.2 M sodium chloride. Any such effect has been shown to be related to the increased activity of inflammatory peptidases. No such effect was observed in this study, because all sugar groups behaved in an almost identical manner (Table II).

The low content of both histamine and serotonin was possibly due to their rapid destruction or their very low concentrations. However, the absence of these inflammatory mediators in the samples studied indicated that the sugar diets and the reduced oral hygiene were not sufficient to cause any severe symptoms of inflammation during the test periods. The use of the sugar substitutes tested was not shown to be periodontologically disadvantageous.

Acknowledgements. The authors wish to extend their thanks to the Finnish Sugar Corporation for the generously provided grant through the Finnish Dental Society. The skilled technical assistance of Mrs. Leila Saarinen and Mrs. Irma Rintanen is gratefully acknowledged.

REFERENCES

Blix, G. 1948. The determination of hexosamine according to Elson and Morgan. ActaChem. Scand. 2, 467—473

Dische, Z. 1955. New color reactions for the determination of sugars, in *Methods of biochemical analyses.* Ed. by *Glick, D.*, Interscience, New York, Vol. II, p 313—355

Hara, K. & Löe, H. 1969. Carbohydrate components of the gingival exudate. J. Periodont. Res. 4, 202—207

Hopsu, V. K., Mäkinen, K. K. & Glenner, G.G. 1966. Formation of bradykinin from kallidin-10 by aminopeptidase B. Nature 212, 1271

Layne, E. 1965. Spectrophotometric and turbidimetric methods for measuring proteins, in *Methods in enzymology.* Eds. by *Colowick, S. P. & Kaplan, N. O.*, Academic Press, London, Vol. 3, p 447

Löe, H. & Holm-Pedersen, P. 1963. Absence and presence of fluid from normal and inflamed gingivae. Periodontics 3, 171—177

Mäkinen, K. K. 1968. Effect of certain chemical compounds on rat liver aminopeptidase B acting on L-arginyl-2-naphthylamine with evidence of the occurrence of a similar enzyme in the liver of human fetuses. Arch. Biochem. Biophys. 126, 803—811

Mäkinen, K. K. & Mäkinen, P.-L. 1971: Effect of sodium chloride on substrate constant and maximum velocity in the enzymic hydrolysis of *N*-L-aminoacyl-2-naphthylamine and *N*-L-aminoacyl-*p*-nitroanilines. Acta Chem. Scand. 25, 969—975

Paunio, K. 1971. On the hydroxyproline-containing components in the gingival exudate. J. Periodont. Res. 6, 115—117

Paunio, K. U., Mäkinen, K. K. & Scheinin, A. 1971. Aminopeptidase B in human gingival exudate as affected by reduced oral hygiene and sugar diet. Acta Odont. Scand. 29, 583—590

Scheinin, A. & Mäkinen, K. K. 1972. Effect of sugars and sugar mixtures on dental plaque. Acta Odont. Scand. 30, 235—257

Shore, P. A., Burkhalter, A. & Cohn, Jr., V. H. 1959. A method for the fluorometric assay of histamine in tissues. J. Pharmacol. Exp. Ther. 127, 182—186

Udenfriend, S., Weissbach, H. & Clark, C. T. 1955. The estimation of 5-hydroxytryptamine (serotonin) in biological tissues. J. Biol. Chem. 215, 337—344