CASEINOLYTIC AND BAEE-ESTERASE ACTIVITY OF SALIVA AND SALIVARY GLANDS OF RATS DURING POSTNATAL DEVELOPMENT

R. D. Barabash and A. P. Levitskii

UDC 612.65'313.1.015.13

The intensity of hydrolysis of casein and of N-benzoyl-l-arginine-ethyl ester (BAEE) by mixed saliva and homogenates of the parotid, submandibular, and sublingual glands of rats was studied during postnatal development. The experiment showed that the highest protease and BAEE-esterase activity was found in the submandibular glands, the main source of their supply to the saliva. After the 15th day of life the proteolytic and BAEE-esterase activity of the submandibular glands begins to increase progressively.

Proteolytic enzymes of the saliva perform several important physiological functions in the oral cavity and lower portions of the digestive tract [3-6, 8].

The present investigation was carried out to study age changes in proteolytic and BAEE-esterase activity of mixed saliva and homogenates of the parotid, submandibular, and large sublingual salivary glands in rats.

EXPERIMENTAL METHOD

Experiments were carried out on 94 male Wistar rats aged 1, 7, 15, 30, 90, 300, and 540 days. Saliva was obtained by stimulation with pilocarpine [2]. Protein was determined by the method of Lowry et al. [7].

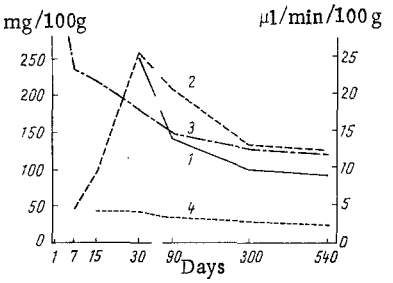

Fig. 1. Age changes in specific salivation (1) and relative weight of parotid (2), submandibular (3), and large sublingual (4) glands in rats. Abscissa, age of rats (in days); ordinate, left: relative weight of glands (in mg/100 g body weight); on right: specific salivation (in μl/min/100 g body weight).

Caseinolytic activity was determined from the rate of hydrolysis of 2% casein solution in 0.1 M Na-K-phosphate buffer, pH 7.6, at 37°C. The incubation mixture consisted of 0.4 ml casein solution and 0.1 ml saliva or gland homogenate. The reaction was stopped with 0.5 ml 10% TCA solution, and the reaction for tyrosine with Folin's reagent was carried out in the supernatant. The extinction was measured after 30 min on the FÉK-60 photoelectric colorimeter at 750 nm. Activity was expressed in milliunits. (One m.u. signifies 1 nmole tyrosine split from casein per minute.)

The BAEE-esterase activity was determined from the rate of hydrolysis of N-benzoyl-l-arginine-ethyl ester. The incubation mixture consisted of 1 ml $1.5 \cdot 10^{-3}$ M BAEE solution in 0.05 M Tris-HCl buffer, pH 8.0, 1.5 ml of the same buffer, 0.25 ml of a solution of soy tripsin inhibitor (0.2 mg in 1 ml 0.85% NaCl solution), and 0.25 ml saliva or homogenate in the appropriate dilution. The change in extinction of the samples during incubation for 15 min was determined in the constant-temperature cells (30°C) of the SF-4A spectrophotometer at 253 nm. Activity was expressed in milliunits. (One m.u. signifies 1 nmole BAEE hydrolyzed per minute.) The results were subjected to statistical analysis [1].

Laboratory of Biochemistry and Radiology, Experimental Division, Odessa Research Institute of Stomatology, Ministry of Health of the Ukrainian SSR. (Presented by Academician of the Academy of Medical Sciences of the USSR A. E. Braunshtein.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 76, No. 8, pp. 65-67, August, 1973. Original article submitted August 14, 1972.

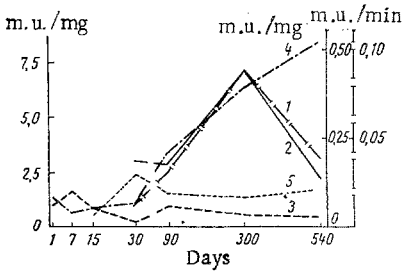

Fig. 2

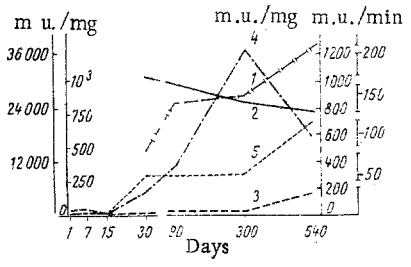

Fig. 3

Fig. 2. Age changes in production (1) and specific activity (2) of proteases of the saliva and parotid (3), submandibular (4), and large sublingual (5) glands in rats. Abscissa, age of rats (in days); ordinate, on left: specific activity (in m.u./mg protein) of proteases of salivary glands; on right: production (in m.u./min of secretion) and specific activity (in m.u./mg protein) of salivary proteases.

Fig. 3. Age changes in production (1) and specific activity (2) of BAEE-esterases of saliva and parotid (3), submandibular (4), and large sublingual (5) glands in rats. Abscissa, age of rats (in days); ordinate, on left: specific activity (in m.u./mg protein) of BAEE-esterases of salivary glands; on right: production (in m.u./min of secretion) and specific activity (in m.u./mg protein) of salivary BAEE-esterases.

EXPERIMENTAL RESULTS

The experiments showed (Fig. 1) that the submandibular glands, whose relative weight in newborn rats is very high (400 mg/100 g body weight), increase in weight progressively more slowly during postnatal development than the total body weight of the animals. The parotid glands, on the other hand, grow at the most rapid rate in the period from birth until the age of 30 days.

The most characteristic feature of the proteases (Fig. 2) and BAEE-esterases (Fig. 3) of the submandibular gland was a sharp rise in their activity which started on the 15th day of life. Since this process results in increased metabolism of proteins, this suggests that the increase in proteolytic activity itself determines the relative atrophy of the submandibular glands with age (Fig. 1). In the parotid glands the activity of these enzymes remains low, at the level characteristic of the newborn animals, throughout life. In the submandibular glands of adult rats BAEE-esterase activity was several thousand times, and protease activity 15 times higher than in the parotid glands. Consequently, the main source of the proteases of the saliva is the submandibular glands. Starting from the 10th month of life, the production and activity of the salivary proteases begin to decrease despite the progressive increase in proteolytic activity of the submandibular glands (Fig. 2). This suggests that with age the formation and accumulation of nonsecreted proteases of the glandulain or cathepsin type increase particularly with age in the submandibular glands of rats. High BAEE-esterase activity was found in the saliva of month-old rats (Fig. 3), although it was still at a low level in the submandibular glands. This can evidently be explained by the low production of inhibitors of BAEE-esterases by the parotid glands which, at this period of postnatal development, are much less well-developed than the submandibular glands.

The increase with age in the production of BAEE-esterases and proteases thus established suggests the compensatory role of this phenomenon in digestive processes and its pathogenetic role in the development of the age atrophy of the parodontal tissues in rats.

LITERATURE CITED

1. E. V. Montsevichute-Éringene, Pat. Fiziol., No. 4, 71 (1964).
2. M. A. Benarde, E. W. Fabian, S. Rosen, et al., J. Dent. Res., 35, 326 (1956).
3. T. O. Ekfors, T. Malmiharju, and P. J. Riekkinen, Biochem. Pharmacol., 16, 1634 (1967).
4. J. A. Guimarais, J. Physiol. (London), 86, 95 (1936).

5. L. C. U. Junqueira and Fava de Moraes, Secretion and Excretion, Berlin (1965), p.36.
6. D. C. Kroeger and J. G. Weatherred, Advances Oral Biol., 2, 31 (1966).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
8. K. Takeya et al., Jap. J. Allergy, 18, 537 (1969).