Effect of age on the activity of rat testicular arginine-aminopeptidase

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Summary. Effects of age on arginine-aminopeptidase activity were investigated in the rat testis. A marked rise of the enzyme activity in 6-week-old rats corresponded to the appearance of spermatids in the later stages of their development.

A histochemical investigation by one of the authors showed that rat spermatids or testicular spermatozoa showed a high activity of arginine-aminopeptidase[1]. This suggests that arginine-aminopeptidase activity may increase with the onset of spermatogenesis if the enzyme plays a physiological role in spermatid development. The present paper describes the change of testicular arginine-aminopeptidase activity with age of the rat, with reference to spermatid development.

Materials and methods. Aminopeptidase activity of rat testis (Wistar strain) was assayed by the method of Felgenhauer and Glenner[1,2]. The methods for histochemical demonstration of aminopeptidase activity were similar to those previously described, with the exception of the coupling azo-dye[1]. As the coupling azo-dye for trapping the enzyme product, β-naphthylamide, Black K salt (ICN Pharmaceutical Inc, Plainview, N.Y.) was employed.

Results and discussion. As shown in figure 1, the activity measured with L-arginine-β-naphthylamide (arginine-aminopeptidase) continued to increase in the testis of 4-week-old rats, while the activity measured with L-leucine-β-naphthylamide (leucine-aminopeptidase) remained at a constant level throughout the age range examined. The level of arginine-aminopeptidase activity in the 6-week-old rats was 2.3 times higher than in the 4-week-old rats.

Histologically, neither spermatids nor spermatozoa were observed in the testis of 4-week-old rats. Spermatogonia, spermatocytes and Leydig cell showed moderate activity of leucine-aminopeptidase and arginine-aminopeptidase in the cytoplasm. Strong activity of arginine-aminopeptidase was observed in the nuclei of some spermatogonia (figs 2 and 3). In the testis of 6-week-old rats numerous spermatids in the latter stages of spermatid development were found as well as mature spermatozoa. These spermatids showed high arginine-aminopeptidase activity but hardly showed leucine-aminopeptidase activity (figs 4 and 5). Some mature spermatozoa showed arginine-aminopeptidase activity. The data obtained indicates that the marked increase of arginine-aminopeptidase activity in the 6-week-old rats corresponded to the appearance of spermatids in the latter stages of their development.

Transition from somatic lysine-rich histone to the arginine-rich or protamine type occurs during the latter stages of spermatid development[3-5]. It depends on the species whether the transition terminates at a protamine or an arginine stage[6,7]. In maturing rat spermatozoa, basic nuclear protein

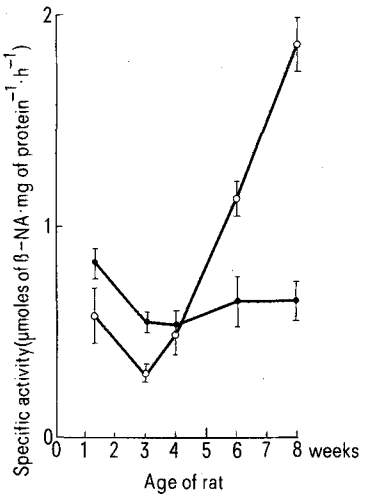

Figure 1. Changes of testicular arginine-aminopeptidase (○), and leucine-aminopeptidase (●) activity with the age of rat. Specific activity is represented as μmoles of liberated β-naphthylamide · mg of protein^{-1} · h^{-1}. 7 rats at 9 days old, and groups of 5 rats at 3, 4, 6 and 8 weeks old were investigated. Vertical lines represent the SD.

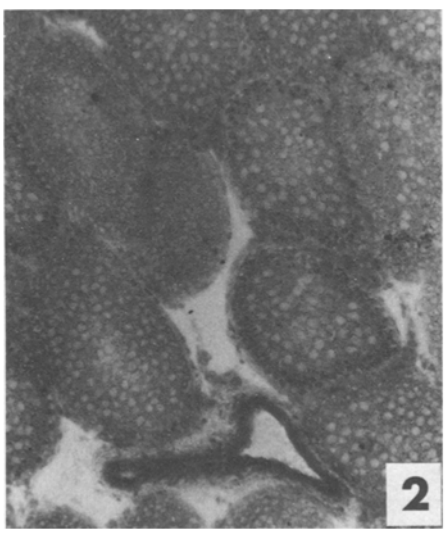

Figure 2. Arginine-aminopeptidase in the testis of 4-week-old rat. The cold acetone-ether treated tissue section (10 μm) made by the method of Takikawa and Matsuzawa[10] was used. Moderate activity in the cytoplasm of Leydig cells, spermatogonia and spermatocytes, and high activity in the nuclei of some spermatogonia were observed. × 80.

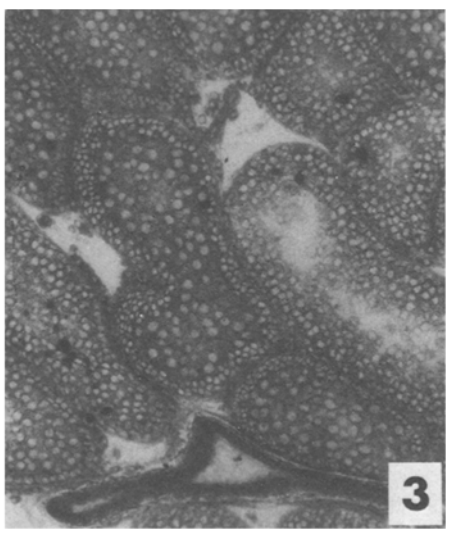

Figure 3. Leucine-aminopeptidase of a section parallel to that in fig. 2. Moderate activity was observed in the cytoplasm of Leydig cells, spermatogonia, and spermatocytes. × 80.

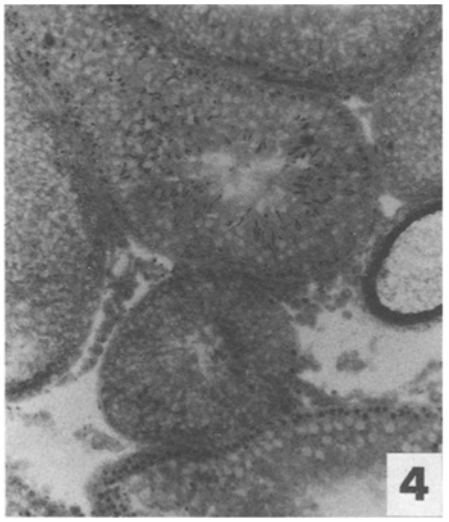

Figure 4. Arginine-aminopeptidase in the testis of a 6-week-old rat. High activity of arginine-aminopeptidase was observed in spermatids in the later stages of their development. × 80.

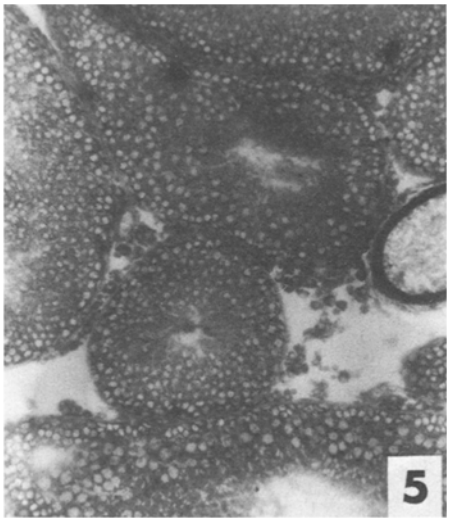

Figure 5. Leucine-aminopeptidase of section parallel to that in fig. 4. Spermatids hardly showed the enzyme activity. × 80.

is protamine rich[8,9]. Protamine, short-chain polyamine, contains mainly arginine and relatively few other amino-acids. These facts and the data obtained strongly suggest that testicular arginine-aminopeptidase is related to the transition of histone occurring through the later development of the spermatid. It is still unknown why arginine-aminopeptidase was higher in the testes of 9-day-old rats than in those of 3-week-old ones in spite of the absence of spermatids. One possible explanation may be that this high activity of arginine-aminopeptidase depends on the activities of different molecular forms or isoenzymes of the enzyme in the spermatid.

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Cleavage of p-nitroanilides of N-acylated tri- and tetrapeptides by alanine endopeptidase from the brush border membranes of rat enterocytes[1]

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Summary. The activity of the alanine endopeptidase from the intestinal brush border was studied using chromogenic substrates of the general fomula Sc-Ala_2-X-pNA, Sc-Y-Z-Ala-pNA and W-Ala_3-pNA respectively. Substrates with C-terminal Leu or Nle are hydrolyzed more readily than Ala-analogues. At least one Ala-residue in one of the positions adjacent to the C-terminus is necessary for the enzyme activity. An N^{α}-substituent has no effect on the activity.

The alanine endopeptidase of the enterocyte brush border has been described in man[3] and in the rat[4]. This enzyme cleaves succinyl-alanyl-alanyl-alanine-4-nitroanilide, previously found to be suitable for pancreatic elastase[5,6], between the 1st and 2nd alanine residues from the C-terminus. In the 2nd step Ala-pNA is split by aminopeptidase (scheme). This enzyme has been found in the kidney, liver, and brain[7]. Its physiological significance, however, has not been explained up to now. The enzyme is similar to the neutral metallo-endopeptidase described by Kerr and Kenny[8] in the kidney, and by Danielsen[9] in the intestinal mucosa. Sogawa[10] compared the catalytic properties of the renal and intestinal enzymes with the use of natural substrates. This study deals with the effect of the substitution in the positions P'_1, P_1, P_2 and P_3 (nomenclature of Schechter and Berger[11]) of the synthetic peptide substrates on the activity of intestinal alanine endopeptidase.

Material and methods. The enzyme preparation containing endopeptidase and aminopeptidase was obtained from solubilized brush border after centrifugation at