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Four aminopeptidases with distinct enzymatic properties in testicular tissue have been found in previous studies (Vanha-Perttula, 1973a). The so-called Enzyme I showed a high specificity for cleaving methionine, isoleucine and valine residues, and resembled an enzyme which has been studied in connection with wound healing (Mäkinen & Raekallio, 1967, 1968, 1969). Enzyme III appeared to have characteristics similar to those described for aminopeptidase B in many mammalian tissues, with a substrate specificity limited to arginine and lysine residues (Hopsu, Mäkinen & Glenner, 1966a, b, c). On the basis of biochemical studies, it has been assumed that Enzyme III is located mainly in the seminiferous tubules and Enzyme I in the interstitial tissue (Vanha-Perttula, 1973a, b). Because it is impossible to establish the exact localization of these aminopeptidases in testicular tissue by purely biochemical methods, this investigation was undertaken to determine whether localization could be achieved with a modified azo-coupling histochemical method.

The guinea-pig was chosen as the test animal, because previous attempts to demonstrate aminopeptidases histochemically in rat testis have been unsuccessful (Nachlas, Monis, Rosenblatt & Seligman, 1960; Dey & Deb, 1973). Three adult male guinea-pigs were used in the present experiment. The animals were killed by incising the aorta under light ether anaesthesia. The testes were removed by dissection immediately after killing the animals. Samples from the testes of each animal were quickly frozen on dry ice, then attached to a specimen holder using distilled water and cut at 16 μm in a cryostat (Ames, model 4552). The temperature of the cryostat was −20°C. For the histochemical demonstration of aminopeptidase activity, a semipermeable membrane-gel combination was used for the incubation of the sections (Huusko, 1974). Because the method is not in common use, it is briefly described.

Visking dialysis tubing was cut into 5 x 5 cm pieces. The pieces were prepared in 0-05% EDTA solution in order to eliminate impurities and heavy metals present in the membrane and then rinsed in distilled water. Incubation vessels were made by cutting up lengths of glass tubing into sections 2.5 cm in length and 2 cm in diameter. The membrane was stretched over one end of the
N-L-arginyl-2-naphthylamine effect on amine substrates has been investigated in Leydig cells. The vessels with the membranes were then placed on a moist glass plate, the membrane being against the glass. The vessels were filled with the warm incubation medium and left to solidify at room temperature for 15 min. The composition of the incubation medium was as follows:

- 0.05 M-phosphate buffer, pH 7.2: 8 ml
- Substrate, in 1 ml solvent: 9 mg
- Fast blue B salt in 1 ml distilled water: 36 mg
- NaCl in 2 ml distilled water to a conc. of 0.2 M: 12 ml

The substrates, N-L-arginyln-2-naphthylamine and N-L-valyl-2-naphthylamine, were obtained from Mann Research Laboratories (New York). Fast blue B salt was obtained from Edward Gurr Ltd (London), Agar from Difco (Detroit) and the semipermeable membrane and Visking dialysis tubing from Medicell (London). Because N-L-valyl-2-naphthylamine is poorly soluble in water, it was dissolved in N,N-dimethylformamide (Merck). The sections were mounted from the cryostat knife onto the membranes by gently but quickly pushing the membrane onto the sections. Before mounting the membrane was dried thoroughly. After mounting, the vessels were immediately incubated at 32°C. The incubation time with N-L-arginyln-2-naphthylamine was 1 hr, but with N-L-valyl-2-naphthylamine it was 2 hr. After the incubation, the vessels with membranes and gels were placed into formaldehyde vapour for 15 min. The membranes were then loosened from the vessels and the part containing the sections was separated with scissors, washed in distilled water, kept in 0.1 M-cupric sulphate solution for 2 min, dehydrated in alcohol, cleared in xylol and mounted on glass slides in Canada balsam and overlaid with coverslips. Control sections were prepared as follows: (a) with the normal gel composition after boiling in water for 15 min, (b) with the gel from which the substrate had been omitted, (c) with the gel from which Fast blue B salt had been omitted, and (d) with the gel stained with azo-dye (free 2-naphthylamine coupled with Fast blue B salt). To demonstrate the effect of chloride ions on enzyme activity, additional sections were incubated with the gel from which NaCl had been omitted. The purpose of this procedure was to discover whether N-L-arginyln-2-naphthylamine was specifically cleaved by aminopeptidase B, which is known to be strongly activated by chloride ions (Hopsu et al., 1966a). Parallel sections from each sample were fixed in 10% neutral formalin for 30 min and stained using the hematoxylin–eosin technique for cytological studies.

With both substrates, the staining was localized specifically to the area of the Leydig cells (Pl. 1, Figs 1 and 2). From the control sections prepared as described above, those from groups (a), (b) and (c) were negative when both substrates were used, and those from group (d) were also negative. When the chloride ions were omitted from the incubation gel and N-L-arginyln-2-naphthylamine was used as a substrate, the sections were negative. Chloride ions had no effect on the staining when the substrate was N-L-valyl-2-naphthylamine.

Because chloride ions were essential for producing any staining when N-L-arginyln-2-naphthylamine was used as a substrate, it is logical to expect...
Fig. 1. Aminopeptidase B activity in the guinea-pig testis. The staining appears exclusively in the area of the Leydig cells. × 64.

Fig. 2. Aminopeptidase activity in the guinea-pig testis when N-L-valyl-2-naphthylamine was used as the substrate. Here also the staining appears in the area of the Leydig cells. × 64.
that, in this study, N-L-arginy1-2-naphthylamine was specifically cleaved by aminopeptidase B, which has been previously demonstrated histochemically in gingival tissue (Mäkinen & Paunio, 1972; Huusko, 1974). The attempts to demonstrate N-L-valyl-2-naphthylamine-cleaving enzymes histochemically have, however, been more or less unsuccessful. According to Vanha-Perttula (1973a), one reason for the failures may be the SH-activation of the enzyme, which makes it vulnerable to the diazo reagents in the histochemical reaction. The method used in the present study demonstrated that when the enzymes are prevented from dissolving into the incubation medium, the histochemical study of N-L-valyl-2-naphthylamine-cleaving enzymes is possible without SH-activation.

Both the enzymes studied were located in the area of the Leydig cells. On the basis of earlier biochemical studies (Vanha-Perttula, 1973a, b), it has been assumed that valine residue-cleaving enzymes were located in the interstitial tissue and arginine residue-cleaving enzymes (aminopeptidase B) were mainly located in the seminiferous tubules. The present study confirms, however, that in the guinea-pig testis aminopeptidase B is primarily localized in the interstitial tissue.

REFERENCES


