

Histochemical Evidence of Aminopeptidase Activity in Rat Pineal Gland

ALTHOUGH various functions have been suggested for the pineal gland¹, very few positive findings have been made concerning this gland^{2,3}. In this communication histochemical evidence is given of a strong enzyme activity in the rat's pineal gland capable of splitting L-leucyl- β -naphthylamide hydrochloride. Recent studies^{4,5} indicate that this activity is actually due to a group of peptidases, including 'leucine aminopeptidase'.

The pineal glands of about 20 albino rats were studied. After removal from the animals the glands were embedded in brain and rapidly frozen. Fresh frozen cold microtome sections were cut at 30 μ . The sections were fixed on coverslips and incubated according to the method of Nachlas *et al.*⁶, using diazotized ortho-dianisidine or diazonium salt of 4-amino-3 : 2'-dimethyl azobenzene as the coupling agents.

The pineal body of the rat exhibited a strong aminopeptidase activity (Fig. 1). The contrast between the staining of the brain tissue and the pineal tissue was very pronounced; when the sections were incubated for 30 min. the brain tissue showed practically no staining at all, whereas the pineal tissue was intensely stained. The enzyme activity of the pineal body was not, however, quite as strong as it was in the rat kidney. The aminopeptidase activity seemed to be evenly distributed throughout the gland; the choroid plexus was infrequently cut together with the pineal body, and it was found to exhibit a moderate enzyme activity.

Pineal bodies of rats of different age groups ranging from 1 to 16 months showed an equal aminopeptidase activity. No sex differences either could be noticed.

Peptidase activity was also tested using ortho-acetyl-5-bromindoxyl as a substrate at an acid medium and diethyl-*p*-nitrophenylphosphate (E600) as an esterase inhibitor⁷. This reaction was positive in the rat's pineal body as well, demonstrating cathepsin-*C*-like peptidase activity.

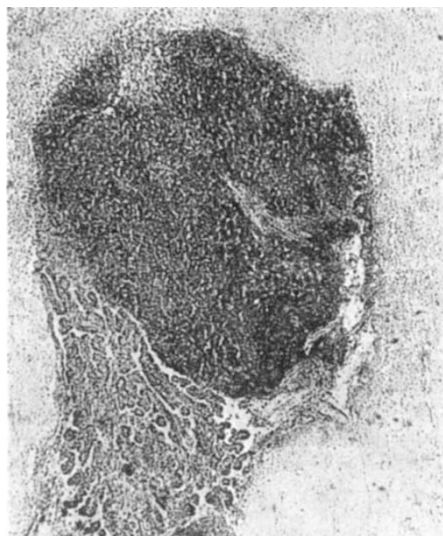


Fig. 1. Leucine aminopeptidase activity in pineal body of a normal adult rat. The choroid plexus beneath the gland shows enzyme activity as well, but the background staining of the brain tissue is due to decomposed diazonium salt (incubation time, 30 min.). ($\times c.$ 40)

So far as the proposed hormone production of the pineal gland is concerned, the present finding may be of interest. Pearse and Tremblay⁸ have recently found an interesting correlation between the hormone production of the rat parathyroid gland and its content of histochemically demonstrable leucine aminopeptidase. The parathyroid hormone, on the other hand, is known to be a mixture of polypeptides. The existence of aminopeptidase activity can be taken to suggest secretion of some agent of a protein nature by the rat pineal gland.

MIKKO NIEMI
M. IKONEN

Department of Anatomy,
University of Helsinki,
Sittavuorenpenget, Helsinki.

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Clearances of Endogenous α -Ketoglutaric and Pyruvic Acid in Man

THE clearances of endogenous α -ketoglutaric and pyruvic acids were investigated in six healthy persons and in ten patients with damaged kidney parenchyma. All persons were confined to bed during the whole experiment. α -Keto-acids were determined after their conversion to corresponding hydrazones which were separated by paper electrophoresis¹.

In all patients with kidney damage the creatinine clearance was lowered. The urine was collected in eight 2-hr. portions during a day and in two 4-hr. portions in the course of a night. (The mean values of clearances of α -ketoglutaric acid and of pyruvic acid was calculated from ten collection periods.)² The blood was deproteinized immediately after venipuncture and the clear supernatant was processed as usual.

The values of both the α -keto-acids in blood varied during a 24-hr. period. The variations averaged ± 15 per cent in the case of α -ketoglutaric acid and ± 20 per cent of values of pyruvic acid. 1.00–2.70 mgm. of α -ketoglutaric acid per 100 ml., and 0.90–1.70 mgm. of pyruvic acid per 100 ml. of urine were usually found. (The concentration was found to be dependent upon diuresis.) Women excreted more of these acids during 24 hr. than men; this difference being accentuated in the case of α -ketoglutaric acid (Table 1).

The mean values of clearances of endogenous α -ketoglutaric and pyruvic acids were higher in women as compared with men (Table 1). In patients with kidney damage the values of both clearances were lowered; the lowest one was found in nephritis of middle degree.

Highest values of clearances were observed between 1 p.m. and 5 p.m., and between 7 p.m. and 9 p.m.

The cause of different concentrations of both α -keto-acids in blood and urine is to be seen in kidneys. The kidney does not only eliminate the α -keto-acids, it participates in their metabolism, too³. The transaminations, oxidative deaminations, and oxidative