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Localization of Tripeptidyl Peptidase I Activity in Different Parts of the Rat Brain

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Tripeptidyl peptidase I is a widespread lysosomal protease, very important for the brain function. Its genetically determined deficiency causes the late infantile form of classical neuronal ceroid lipofuscinosis – a serious neurodegenerative disorder, connected with severe symptoms and early death at puberty. The enzyme is known to be active in many neuronal types in the brain. Its distribution in the cerebral and cerebellar cortex medulla oblongata and cervical part of the spinal cord of laboratory animals has been well described using immunohistochemistry and also, enzyme histochemistry. The enzyme locations in other parts of the brain have not been elucidated yet. In the present paper we describe TPP I activity localization in mesencephalon, thalamus and pons of the adult rat brain using the enzyme histochemical method, recently developed by us.

Key words: tripeptidyl peptidase I, rat brain, enzyme histochemistry.

Introduction

Tripeptidyl peptidase I (EC 3.4.11.19) is a lysosomal peptidase cleaving off tripeptides from the free amino-terminal of polypeptides. The enzyme is widely distributed in human and mammalian organs and tissues as well as in the central nervous system (CNS) [5, 6 ,7]. Its functions are not clear yet, but it is known to take part in the hydrolysis of a great number of neuropeptides thus altering their activity and/or receptor specificity [3, 4, 11]. The presence of TPPI activity in the CNS is crucial for the neuronal functions. Its genetically determined deficiency causes the severe neurodegenerative disease late infantile neuronal ceroid lipofuscinosis (LINCL) [9]. Recently, a mouse model of LINCL has been developed by a directed disruption of CLN2 gene encoding TPPI [10]. The animals suffer a similar neurologic disease as humans and this model opens new possibilities for the study of LINCL. The enzyme distribution in the CNS of laboratory animals (rats and mice) as well as in humans is usually studied using immunohistochemical methods. TPPI localization in the neurons of cerebral and cerebellar cortices and also in the neurons of the hypoglossal nerve nuclei and the cervical part of spinal cord has been established both by immunohistochemical and enzyme histochemical

methods [2, 6]. However, the enzyme locations in the other parts of the brain are not studied yet.

The aim of the present paper is to determine TPPI localization in adult rat thalamus, mesencephalon and pons using the enzyme histochemical method, recently developed by us.

Materials and Methods

Adult Wistar rats of both sexes were decapitated in deep anesthesia. Thalamus, mesencephalon and pons were extracted and fixed in 0,067 M phosphate buffer, pH 7.0 containing 4 % sucrose for 18 h at 4°C. Then, the brain parts were washed with 30 % aqueous solution of sucrose supplied with 1 % gum Arabic for 48 h at 4°C. Finally, the samples were frozen in liquid nitrogen. Tissue sections (10 μm) were cut on cryotome Reichert Jung 2800 (FRG) and mounted on gelatinized glass slides. They were covered by celoidine (1% in acetone : diethyl ether : absolute ethanol 4:3:3) for a minute at room temperature just prior use.

Localization of TPPI activity in the tissue sections: This was performed as described before [1]. Briefly, the sections were incubated in a substrate medium consisting of 0.5 mmol enzyme substrate Gly-Pro-Met-1-anthraquinonyl hydrazide (synthesized after Dikov et al. [1]) and 0.5 mg/ml 4-nitrobenzaldehyde in 0.1 M acetate buffer, pH 4.5 for 70 min at 37°C. Then, they were post-fixed in 4 % neutral formalin for 15 min at room temperature. The sections were stained by haematoxylin according to the classical methods of histology and embedded in glycerol/gelatin.

Histochemical controls: Control sections were incubated in 0.1 M acetate buffer, pH 4.5 containing 1 μM inhibitor Ala-Ala-Phe-chloromethyl ketone (Bachem, Switzerland) for 45 min at room temeperature. Then, they were transferred to the full substrate medium supplied with 1 μM inhibitor and incubated for 70 min at 37°C. After the incubation, they were treated as the other sections.

All the sections were studied under the microscope LeicaDM50008 (New York, USA).

Results and Discussion

Most of the proteases are interchangeable enzymes, i.e. if a protease activity is defective or lacking, its functions are taken up by other proteases. However, this is not the case with TPPI. Mutations in the gene encoding TPPI (CLN2) are known to cause the LINCL disease [9]. Morphologically, LINCL is connected with a coupling of autofluorescent non-degraded material (lipopigment) in the lysosomes in CNS and some peripheral organs (liver, kidneys, etc.) [8]. The clinical symptoms are such as myoclonal jerks, epilepsy, successive visual loss and early death at puberty. They are brought about by a profound neuronal dysfunction and a subsequent loss of neurons in the CNS. That is why TPPI is believed to be a crucial enzyme for the neuronal function. Recently, a mouse model of LINCL has been developed by a directed disruption of CLN2 gene [10]. Therefore, it is important to elucidate the precise enzyme locations in the CNS of laboratory animals as well as the levels of its activity in different brain regions. The enzyme activity in the cerebral cortex, cerebellar cortex, medulla oblongata and the cervical part of the spinal cord has been documented using both immune- and enzyme

histochemistry. However, the enzyme locations in the other parts of the brain are not studied yet. That is why we decided to investigate TPPI localization in the mesencephalon, pons and thalamic region using the histochemical method recently developed by us. In the rat mesencephalon, the enzyme was highly active in the neurons of nucleus ruber (Fig. 1 A) and nucleus oculomotorius (Fig. 1 B) as well as in the neurons of substantia nigra (not shown here). In the thalamic region, the enzyme activity was visibly much lower than in other studied brain areas (Fig. 1 C and inclusion). The enzyme reaction was visible both in medial and lateral nuclei but was presented by a very small number of positive granules. In the pons, high enzyme activity was detected in all the neurons of the gray matter (Fig. 1 D).The control sections incubated in the presence of TPPI specific inhibitor Ala-Ala-Phe-chloromethyl ketone showed a lack of the final product of enzyme reaction to prove the specificity of this study. Obviously, TPPI is active also in the neurons of rat mesencephalon, pons and thalamus. The significance of the lower enzyme activity in the thalamic region is not known yet and might be a subject of farther investigations.

In conclusion, the histochemical results of TPPI distribution in normal adult rat brain areas presented here can be of value for the future studies on the enzyme importance for the neuronal functions in different brain parts as well as for the studies of LINCL using animal models of the disease.

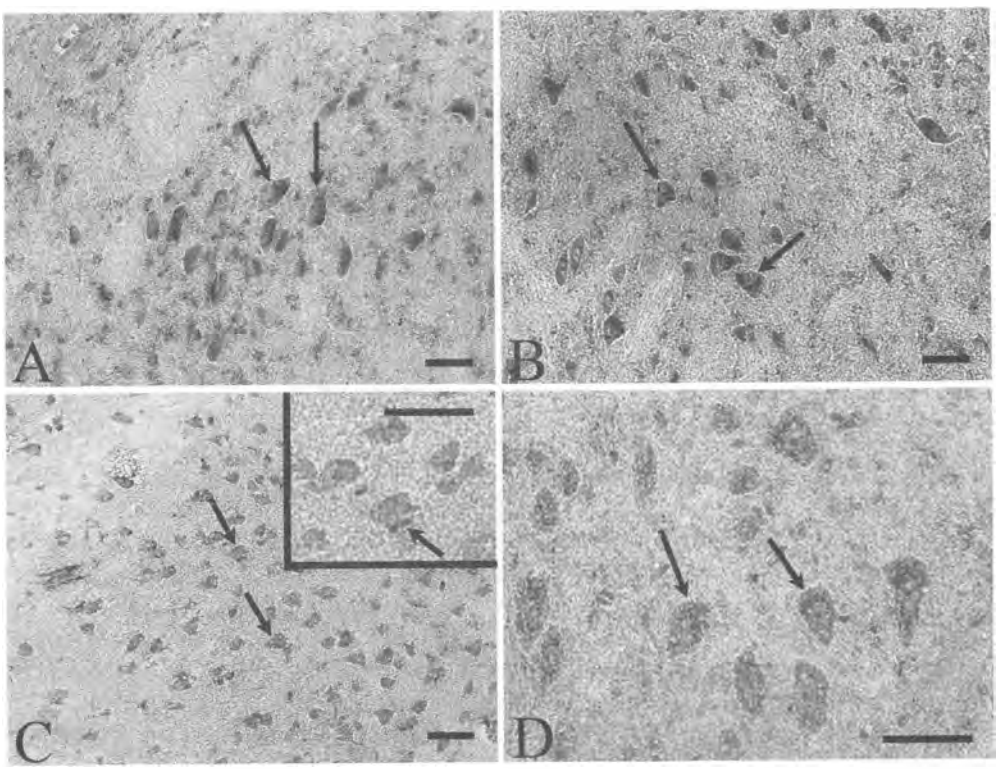

Fig.1. Localization of TPPI activity in different parts of adult rat brain. High enzyme activity (arrows) in the neurons of nucleus ruber (A) and nucleus nervus oculomotorius (B) in the mesencephalon. Lower TPPI activity (arrows) in the thalamic neurons (C). Substantial enzyme activity (arrows) in the pons neurons (D). Scale bars = 50 μm.

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