

Comparative Study of the Activity Levels and Localization of Tripeptidyl Peptidase I in Rat and Mouse Brain

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Tripeptidyl peptidase I is a lysosomal protease, crucial 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. Since most of the brain diseases are now studied using animal models, it is important to identify the enzyme locations and activity levels in healthy laboratory animals' brain regions. However, TPPI locations and activity levels in mesencephalon, thalamus and pons are still largely unknown. In the present paper we determine the enzyme activity levels and localization pattern in the above three brain regions of healthy adult rats and mice. The results show species differences in TPPI activity levels. All the studied types of neurons show high enzyme activity in both species. Those results would be important in view of the use of animal models for studying neurodegenerative disorders.

Key words: tripeptidyl peptidase I, enzyme histochemistry, enzyme kinetics, central nervous system.

Introduction

Tripeptidyl peptidase I (TPPI, E.C. 3.4.14.9) is a lysosomal enzyme – a protein product of the *cln2* gene, mutations in which are known to cause the recessive neurodegenerative disease late infantile neuronal ceroid lipofuscinosis (LINCL) or Jansky-Bielschowsky disease [9]. LINCL is connected with pathological accumulation of autofluorescent lipopigment in the neurons and photoreceptor cells leading to their degeneration and progressive loss [11]. Recently, a mouse model of LINCL has been established [10] and used for testing different therapeutic strategies [8]. On the other hand, changes in TPPI activity levels in human brain have been found in many diseases like Alzheimer disease, Down syndrome, other forms of neuronal ceroid lipofuscinosis, sclerosing panencephalitis and brain infarctions [5]. Some of the above disorders are also studied using animal models, particularly mice and rats. Therefore, it is important to identify the enzyme activity levels and localization pattern in normal rats and mice brains for comparative purposes. TPPI activity studies have been performed in cerebral cortex, cerebellar cor-

tex and spinal cord of adult rats and mice using biochemical assays [2, 6], immunohistochemistry [6] and enzyme histochemistry [3]. However, the enzyme localization and activity levels in rat or mouse thalamus, mesencephalon and pons are largely unknown.

The aim of the present study is to determine TPPI activity levels and cell localization in the normal adult rat and mouse thalamus, mesencephalon and pons. The results can be useful for the study of different brain diseases using mammalian models.

Materials and Methods

Adult Balb/C mice (3-month-old) and Wistar rats (5-month-old) of both sexes were decapitated in deep anesthesia.

Enzyme assays in tissue homogenates. Mesencephalon, thalamus and pons were extracted and the enzyme assays were performed exactly according to the procedure described in [2]. Briefly, the brain parts were homogenized in lysis buffer (1% Triton $\times 100$ and 0.15 M NaCl, dissolved in 0.05 M sodium acetate buffer, pH 4.5) on ice. The lysates were diluted with 0.4 M sodium acetate buffer, pH 4.5, supplied with 4 mM EDTA. After centrifugation at 4000 rpm, supernatants were diluted with 0.2 M sodium acetate, pH 4.5, containing 2 mM EDTA and the protein content was determined by measuring the samples absorption at 260 and 280 nm on a spectrophotometer Speckol 1500 (Analitjk, Jena) and calculations as described by Dawson et al. [4]. The activity of TPPI in tissue homogenates was assayed in incubation solutions, containing 0.5 mM substrate alanyl-alanyl-phenylalanine-p-nitroanilide (AAF-pNA, Bachem-Switzerland) at 37 °C. Aliquots were collected every 15 minutes, in which the reaction was stopped with equal volume of 0.1 M chloroacetic acid in 0.1 M sodium acetate, pH 4.4. Absorption of the samples at 405 nm was measured spectrophotometrically against a control of freshly prepared incubation solution, in which the reaction was immediately stopped as above. The results were statistically estimated by regression analysis and curves showing the time-dependence of the absorption at 405 nm were built by means of Sigma Plot 9.0. One unit of enzyme activity was defined as the amount of enzyme liberating 1 μ M product – *para*-nitroaniline (pNA) per minute per 1 mg protein at 37 °C.

Enzyme histochemistry. 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 μ m) 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 before use. The enzyme localization was performed as described previously [1]. Briefly, the sections were incubated in a substrate medium consisting of 0.5 mmol enzyme substrate Gly-Pro-Met-(1-anthraquinonyl hydrazide) (GPM-AH), 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 μ M inhibitor Ala-Ala-Phe-chloromethyl ketone (AAF-CMK) (Bachem, Switzerland) for 45 min at room temperature. Then, they were transferred to the full substrate medium supplied with 1 μ M 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 Leica DM5000B (New York, USA).

Results

TPPI biochemical assays revealed high enzyme activity in the three studied brain regions of both mice and rats (**Fig. 1**). The enzyme activity per mg protein in the mice brain decreased in the following order: mesencephalon > thalamus > pons, whereas in the rat brain this order was different: thalamus > mesencephalon > pons.

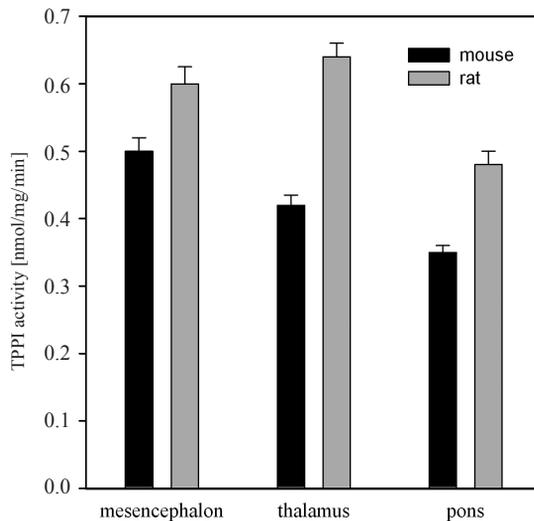


Fig. 1. Biochemical analysis of TPPI activity in rat and mouse brain regions using the substrate AAF-*p*NA in tissue homogenates. Each value was obtained as a mean of five separate experiments using adult animals of the respective species

Histochemical studies showed that TPPI was highly active in the neurons of both nucleus ruber (**Fig. 2A, B**) and substantia nigra (**Fig. 2C, D**) in mesencephalon of the two species. The enzyme activity was visualized as numerous orange-brown granules filling the cell cytoplasm. TPPI-positive granules were visible in the glial cells as well. All the neurons of the mouse (**Fig. 3A**) and rat (**Fig. 3B**) thalamic region were also highly positive for the enzyme as well as the neurons of the pons nuclei of the gray matter (**Fig. 2C, D**). In the thalamus and pons the enzyme activity was also visualize in the form of dark granules, which corresponds to the lysosomal localization of TPPI. Similarly high reaction for TPPI was observed in the glial cells too.

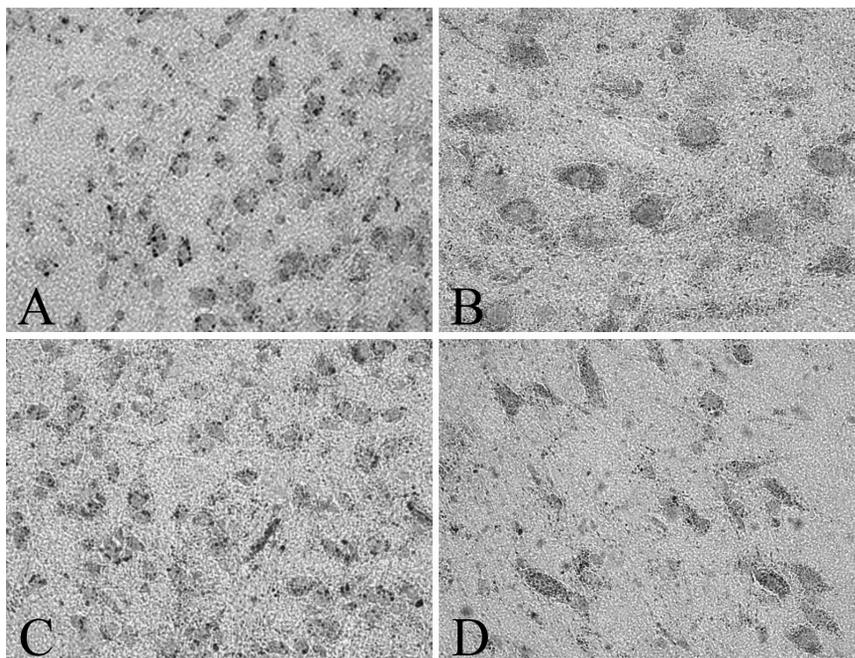


Fig. 2. Localization of TPPI activity in mouse (A, C) and rat (B, D) mesencephalon. High enzyme activity in the neurons of nucleus ruber (A, B) and substantia nigra (C, D). 400 ×

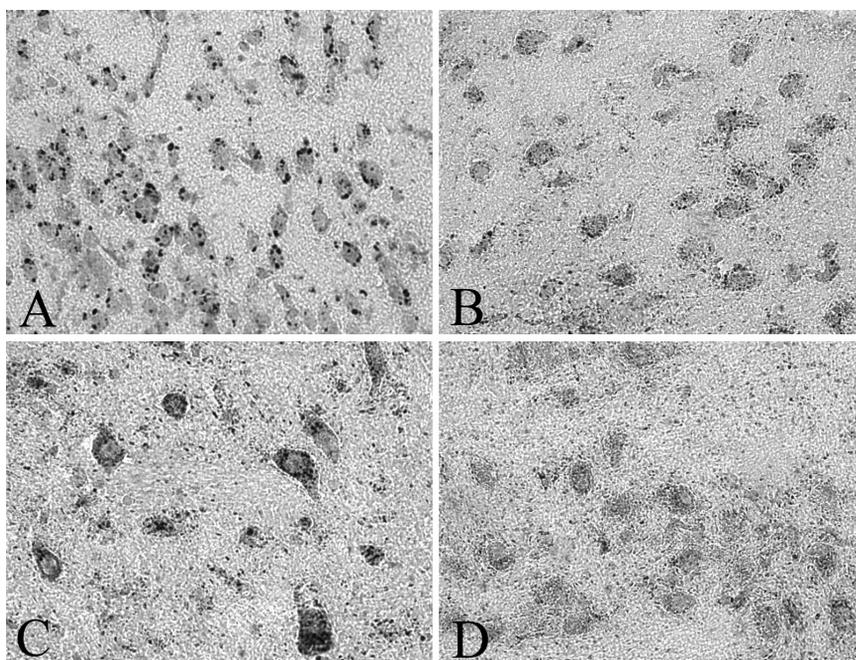


Fig. 3. Localization of TPPI activity in mouse (A) and rat (B) thalamus as well as in mouse (C) and rat (D) pons. High enzyme activity in the thalamus' (A, B) and pons' (C, D) neurons of nuclei of the gray matter. 400 ×

Discussion

TPPI activity is vital for the neuronal functions. The genetically determined enzyme deficiency leads to the development of the recessive neurodegenerative disease LINCL. LINCL is characterized by coupling of undegraded material in the lysosomes and lysosomal dysfunction, enhanced mitochondrial fragmentation and subsequent neuronal death. Clinical symptoms include severe epileptic seizures, ataxia, mental deterioration and progressive visual failure resulting in an early death in puberty [7]. The recently developed mouse model of LINCL [10] opens new possibilities for finding therapeutic approaches for the treatment of this serious genetic disorder [8]. On the other hand, many other brain diseases, also studied by mammalian models are connected with abnormally high or low TPPI activity levels [5]. In that respect, it is important to identify both normal enzyme activity levels and normal enzyme locations in all the brain regions of mostly used laboratory animals, i.e. rats and mice.

In the present paper we report TPPI activity levels and tissue distribution in three brain regions of rats and mice – mesencephalon, thalamus and pons. The enzyme in those brain parts have not been studied thus far. Our results show high enzyme activity levels. However, whereas in mouse the highest enzyme activity per mg protein was detected in the mesencephalon, in rat TPPI activity was most elevated in the thalamic region. This difference between species should be kept in mind when interpreting the results of studies using mouse and/or rat models for different disorders. On the other hand, the enzyme distribution is largely similar in the neurons of the two species. The enzyme was highly active in all the studied types of neurons as well as in glial cells of the three brain regions. This result points out at the crucial role of TPPI in rat and mouse brain as well.

In conclusion, the results presented here would be important in view of the use of animal models for studying LINCL as well as other neurodegenerative disorders.

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