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Morphological Analysis of Brain Microanatomy via Lycopersicon Esculentum Histochemistry

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The description of the brain microanatomy always depends on the development of the histological and histochemical methods of coloration. The specificity of each histological or histochemical procedure limits the number of the visualized structures in the tissue. Thus, in the central nervous system (CNS) as results of the performing of *Lycopersicon esculentum* lectin histochemistry on cryostat and paraffin sections could be found marked two different types of object: lectin(+) cells and lectin(+) non-cellular bigger structures.

In the present study we describe local appearance, tissue distribution and visual topography and identity of the *Lycopersicon esculentum*(+) tissue structures in adult hamster brain. Briefly, this investigation is a test for the very contested between the neuromorphologists specificity of the staining histochemical procedure with *Lycopersicon esculentum* lectin.

Key words: TL (tomato lectin) histochemistry, brain microvasculature microglia.

Introduction

Now many assays are available to monitor the morphology of the CNS microanatomy. Some of them are very specific and more or less sensitive in the visualization of different brain structures. In the last two decades in brain marking Lycopersicon esculentum is used. In the scientific studies Lycopersicon esculentum positive (+) structures are known usually as TL(+) objects (by a terminological abbreviation of *tomato lectin*). Nevertheless, the application of a histochemical procedure with Lycopersicon esculen-

tum lectin in golden Syrian hamster's brain we found considerably detailed picture of the cellular and vascular structures. Previously, we have demonstrated that the generality of the positive to TL structures in the hamster brain really represents transversally cut small capillary vessels [6]. But in several CNS zones due to the Lycopersicon esculentum affinity for poly-N-aceryl lactosamine sugar residues [11] this lectin binds to microglial population in CNS. During the multidirectional searches for the microglia visualization and imaging in the last decade of the 20th century TL was assumed for long period as more or less specific marker for microglial cells in mammal brain as result of the abundance of the above cited residues [1]. Recently the interest to the microglia histochemical imaging rises because microglial population from the cells in mammal's brain is firmly established as a key cellular element in the CNS. They are recognized to serve as brain and spinal cord's innate immune system [9]. The known markers for microglia are cell-type specific and they do not label other glia or neurons [4]. Today, we have a number of well-established markers for microglial cells in mammal's brain. But from them only single specific molecular markers do not label peripheral macrophages or other micro anatomical components [8]. At the same time this controversy is not real reason for the abandonment of several markers for coloration in contemporary brain histochemistry. Some of them as above-mentioned Lycopersicon esculentum lectin continue to be used for microglial identification in CNS [10].

In our study by the use of the dual histochemical specificity of the Lycopersicon esculentum lectin we try to evaluate the hamster brain microanatomy.

Material and Methods

Animals: Adult five-week-old female outbred golden Syrian hamsters – Mesocricetus auratus (17 animals) were used as a source for obtaining of healthy brain tissue.

Histological procedure: The brains were fixed in Carnoy's solution at room temperature overnight. Serial transversal sections 5-7 μ thick were obtained from selected levels using Leica paraffin microtome after embedding in paraffin.

Lycopersicon esculentum lectin histochemistry: Commercially available biotinilated – Lycopersicon esculentum lectin (VECTOR Labs, Cat.No. B-1175) diluted in freshly prepared working solution of 10 μg/ml PBS buffer (phosphate buffer saline), enriched with $CaCl_2$ (1mM), $MgCl_2$ (0.1 mM) and stabilized with Natrium azide (0,08%) was used for histochemical procedure. Recommended working dilution was applied on dewaxed and rehydrated paraffin sections from both healthy hamster brains and after blocking of the endogenous peroxydases on tissue sections with 2,5% H_2O_2 in methanol.

Incubation of the section with Lycopersicon esculentum lectin was performed at 20 °C for 2 h followed by application of ABC reagent kit and DAB substrate kit for peroxidase. The rinsing buffer (phosphate buffer saline) during the whole procedure was enriched with $CaCl_2$ (1mM) and $MgCl_2$ (0.1 mM).

Controls: Whole staining procedure without lectin.

Studied brain regions: Cortex, thalamus, cerebellum.

Microscopy: Light microscopy and interferential contrast microscopy (Nomarski optics).

Results

Previously in our investigations is demonstrated that in healthy hamster CNS Lycopersicon esculentum lectin histochemistry reveals different kinds of positive objects – ones with determined cellular shape bodies, and others – obviously non-cellular elements belonging to the brain microanatomy [1]. The first ones – TL(+) cells possess very irregular shapes with ramified and amoeboid morphologies. Elongated cells with several processes and less or more rounded cells are labelled generally. These cells are structurally absolutely identical to microglia and are seen abundantly in the hamster brains (Fig. 1).

Intriguingly, we observed great variations in the forms of the non-cellular TL(+) objects when samples of the brain tissue were taken from different zones. For example in the study of the hamster cortex microanatomy (using proposed histochemical pro-

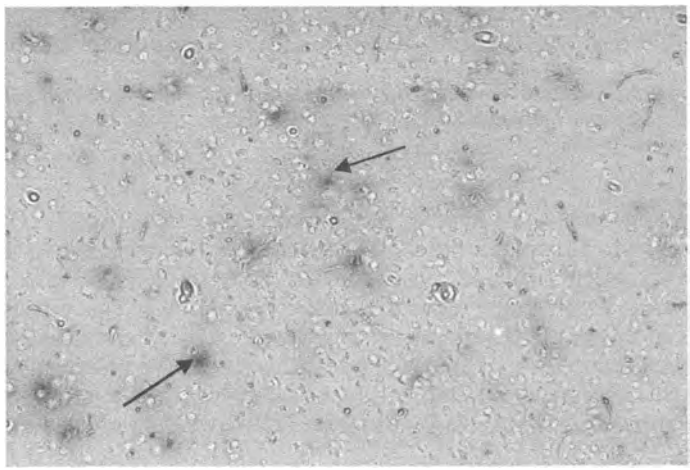

Fig. 1. TL(+) cellular objects with ramified and amoeboid morphologies (arrows) and smaller in size non-cellular objects between them representing a map of the brain microvasculature. Cortex, × 160

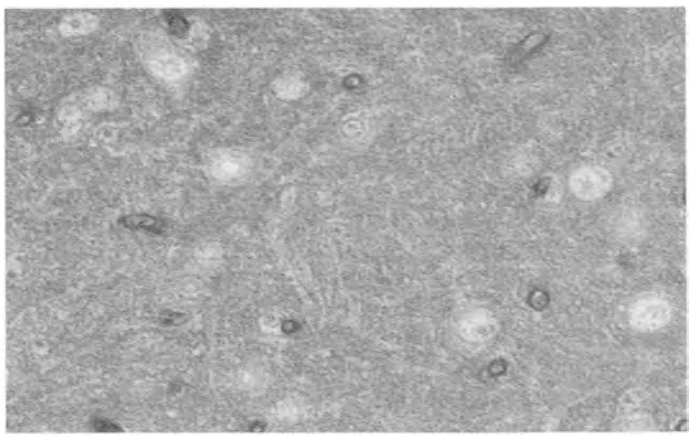

Fig. 2. Different forms of marked by TL histochemistry structures in Thalamus, × 400

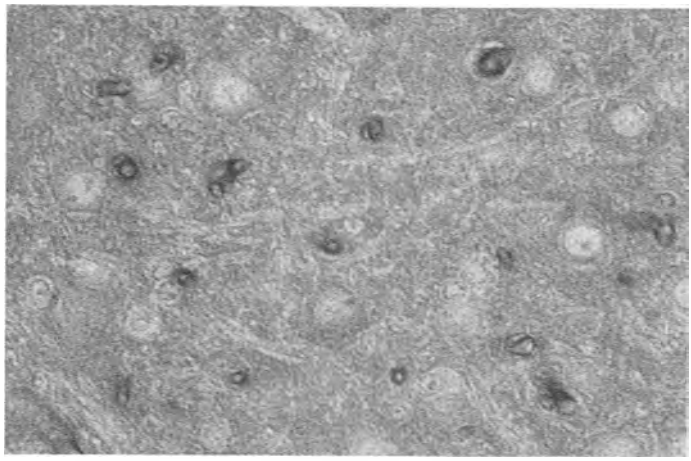

Fig. 3. TL histochemistry: tissue sections from the hamster cerebellum demonstrate convincingly that non-cellular TL(+) objects in CNS are microvessels, × 400

cedure) shows very peculiar forms of the marked structures (Fig. 2). Big rounded or prolonged positive to TL objects are situated abundantly everywhere in the brain tissue and they are very often localized closely to groups of unlabelled cells. Assuming the abundance of these non-cellular structures, their morphologic characteristics and their distribution as loose-textured network everywhere in studied zones, we determine that these objects are components of the brain microvasculature – transversally or longitudinally cut. On the other hand, all obtained by us Nomarski optics images in the hamster cerebellum demonstrate convincingly that non-cellular TL(+) objects in CNS are microvessels (Fig. 3).

Discussion

The morphologic analysis of the brain microanatomy via Lycopersicon esculentum histochemistry made by us aims to help and perfect the brain mapping in vertebrate species. In this mapping the markers are crucial for the identification of cells and non-cellular components of the CNS structure. In this study we show various morphological signatures of the brain microanatomy using a simple histochemical method for visualization of these objects in CNS. The studies on vertebrate brain structure and especially the construction of the CNS map of this system are full for many years of historical controversies surrounding their achievement. A clear retardation in this field could be explained by the slower introduction of many new histochemical and immunohistochemical markers and procedures.

Our investigations of the brain microanatomy by Lycopersicon esculentum lectin in the healthy adult hamster contribute to this mapping. Brain TL labelling now reveals readily specific distribution, morphology and morphometry of the microglial population [5]. On the other hand, the use of this marker in neuromorphology represents a suitable methodology for generation of structural and even 3D maps of vascularized brain parenchyma [7]. As evidenced scientific publication from the last five years the dualism in the specificity of the TL histochemistry in CNS persists till now but many authors

continue to use Lycopersicon esculentum lectin histochemistry as specific microglial marker [2, 3].

The final result of our study is a new specific light microscopic image of the brain microanatomy suitable for early registration of the morphological signs in CNS during the development and in some pathology of the brain.

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