Aspartatergic Pyramidal Neurons of Betz in the Cat Motor Cortex

V. E. Okhotin and S. G. Kalinichenko

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Field 4 of the cat motor cortex was histochemically tested for aspartate aminotransferase (AST). High activity of the enzyme was detected in 16-18% of large and giant Betz's pyramidal neurons of layer V and in the synaptic terminals on their bodies and dendrites.

Key Words: *aspartate aminotransferase; motor cortex; pyramidal neurons of Betz*

Chemical mapping of pyramidal neurons of the neocortex is the key to the understanding of the information processes in the brain and of neuronal interactions. The mediator specialization of giant pyramidal neurons of Betz as the "command" neurons of the neocortical motor area are particularly important. Published data on their neurochemical type are contradictory and incomplete. For example, these cells were reported to utilize acetylcholine and glutamate for the nerve pulse transfer in the brain cortex of humans [1,2] but not of animals [3].

This study was aimed at locating aspartate aminotransferase (AST, EC 2.6.1.1) producing the stimulating mediator L-aspartate in the giant pyramidal neurons of field 4 of the cat motor cortex.

MATERIALS AND METHODS

The brain of 5 adult cats was examined. The animals were anesthetized with ether. The motor cortex was isolated with a razor blade and immediately frozen in a cryostat. AST was detected histochemically by a previously described method [9] with modifications [6]. Some sections of slices were stained after Nissl. Sections 25 μ thick were cut in the frontal plane across all layers of the cortex and incubated for 15 min at 37°C in medium containing 0.1 M HEPES buffer (Serva, pH 7.4), 20 mM L-aspartate (Sigma), 4 mM α-ketoglutarate (Sigma), 50 mM imidazole (Fluka), 6 mM of lead nitrate, and 2% polyvinyl alcohol (Merck). After the incubation, the sections were washed with warm distilled water, dehydrated, and routinely embedded in balm. In the control experiments, 20 mM malate, a specific inhibitor of AST, was added to the medium, so the sections were not stained. Pyramidal neurons reacting to AST were counted in layer V of the fourth field and expressed as percentage of all cells stained after Nissl.

Histology Department, Vladivostok State Medical Institute

RESULTS

A brown precipitate formed in the cell cytoplasm as a result of histochemical reaction to AST. The intensity and consistency of this precipitate varied depending on the enzyme activity. The distribution of aspartatergic neurons was uneven. Their bulk was found in layers V and VI of the cortex, whereas in layer III, where the number of pyramids is the greatest, aspartatergic cells were rather scarce. The majority of pyramidal cells in layer III do not react to AST.

In layer V, 16-18% of large and giant pyramidal neurons were revealed by the AST test. This population consisted of cells with high, medium, and low enzyme activity in large lumps of black-brown precipitate more or less evenly distributed on the surface of the perikaryon formed in the neurons of the first group. In the perinuclear area of the cytoplasm, the precipitate granules fused to form a homogeneous mass and fill the proximal portions of apical and basal dendrites (Fig. 1). After bifurcation of these dendrites, the precipitate became less

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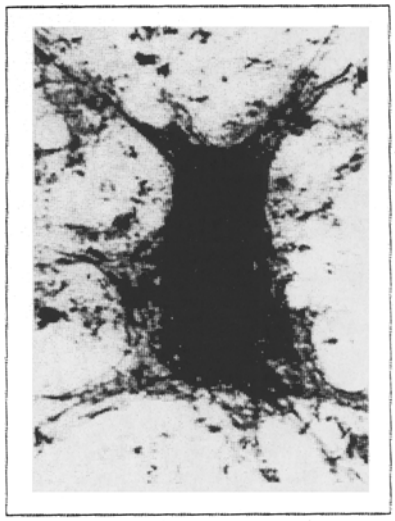

Fig. 1. Pyramidal neuron of Betz with a high AST activity. Here and in Figs. 2 and 3: method [9] with modifications [6]. ×400.

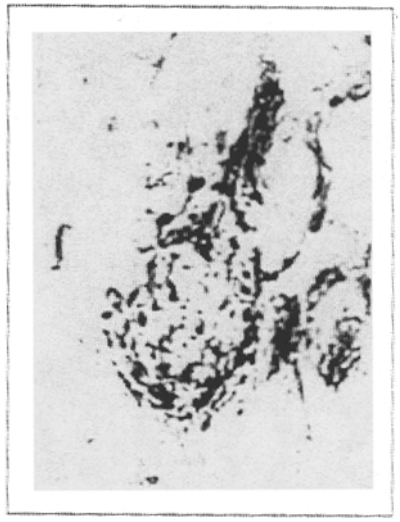

Fig. 2. High activity of AST in the synaptic terminals on the body and apical dendrite of pyramidal neuron. ×400.

dense and looked like small dots outlining thin dendrite branches. Neurons with high AST activity amounted for 30-40% of positively stained cells and were represented only by giant-cells of Betz. All AST-positive cells of Betz had an axon branching from the cell base or from the basal dendrite. The axon ran perpendicular through layer VI, and its short segment was seen in the white matter.

Cells with medium enzyme activity amounted to about 62-64% of the large pyramid population. The precipitate was light-brown and more dense in the center of the cell body, forming an empty space near the nucleus.

Pyramidal neurons with a low AST activity represented a relatively small population of aspartatergic neurons. The reaction product was powder-like; it evenly stained cell perikaryon and rarely varied in density in dendrites. The weak staining of these cells permits the detection of numerous synaptic terminals on their surface, which were seen as solitary "buttons" or necklace structures (Fig. 2). Such a convergence may also occur in intensely stained pyramids, near which AST-positive nerve fibers always formed an irregular network. However, massive fields of the marker precipitate may mask the loci of synaptic terminal contacts, thus precluding their precise identification.

The AST-containing pyramidal neurons tended to form nests or modules generally including one giant neuron of Betz, 2-3 large pyramidal cells with high and medium AST activity, and several smaller cells with or without small amounts of the precipitate (Fig. 3). The effect of neurons of Betz on the stimulating activity of the module is likely to be the principal factor, determining the major mediator balance of aspartatergic effectors.

The fact that the enzyme localization may not coincide with the activity of the neurotransmitter aspartate pool and label the metabolic pool [7] should be taken into account upon histochemical identification of AST. Therefore, it is necessary to distinguish between synaptic and nonsynaptic fractions of the enzyme, which determines the specificity of histoenzymologic staining. Obviously, in our preparations pyramidal neurons were labeled at random. Their contribution to the transmission of corticofugal projections is comparatively small. Presumably, other neurotransmitters (acetylcholine, glutamate, or neuroactive peptides) are involved in signal transmission in the overwhelming majority of pyramidal cells [5,8,10]. Meanwhile, it was reported that 66% of pyramids in layer V of the rat brain visu-

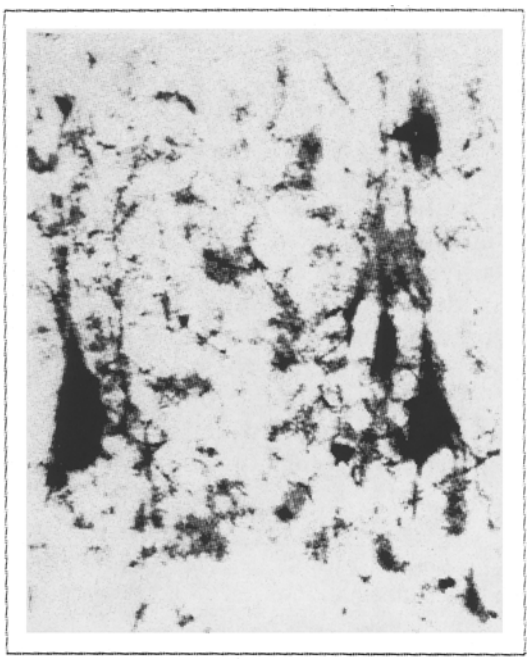

Fig. 3. Aspartatergic pyramidal cells of layer V. ×200.

al cortex contain immunoreactive aspartate [4]. In this case, sera may react with both neurotransmitter and metabolic aspartic acid pools, which accounts for the high values [4].

The fact that nerve fibers, synaptic terminals, and axons of giant cells of Betz stain positively for AST indicates that the aspartate is also produced in these structures.

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Ultrastructural, Radioautographic, and Morphometric Analysis of Gastric Mucosa in Chronic Gastritis

V. V. Omigov, D. L. Nepomnyashchikh, and G. A. Lapii

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Biopsy specimens of gastric mucosa from patients with chronic gastritis are examined. The dynamics of structural changes occurring during the development of chronic inflammation is demonstrated and the ultrastructural changes in the mucosa cell populations are described, which together with the radioautographic analysis of biosynthetic reactions and morphometric data characterizes the complex structural and functional rearrangements in the gastric mucosa.

Key Words: *chronic gastritis; gastric biopsy; electron microscopy; radioautography; morphometry*

Gastritis, a process with polymorphic structural manifestations, is the most typical reaction of gastric mucosa in disease. The polymorphism results primarily from the specific combination of destructive and adaptive components [2,3,11,13,14]. The interpretation of structural changes in the gastric mucosa has markedly changed after the investigation into its normal function during eating and between digestion [4,9], which led to the concept of functional morphology of the stomach in health and disease.

Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk

In this study biopsy samples of the gastric mucosa obtained during chronic inflammation were studied with the use of complex morphometrical analysis and *in vitro* radioautography.

MATERIALS AND METHODS

The morphology of 230 specimens of the mucosa from the fundal and pyloric regions of the stomach obtained by fibrogastroscopy was analyzed. Light microscopy of paraffin and semithin sections, transmission and scanning electron microscopy, and incubation of biopsy specimens with ^{3}H-uridine and ^{3}H-thymidine to estimate RNA and DNA synthe-

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