Histochemical Detection of 3β-Hydroxysteroid Dehydrogenase in Neurons of Rat Brain

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Here we present the results of histochemical detection of 3β-hydroxysteroid dehydrogenase in neurons of the neocortex, cerebellum, and brainstem. The positive reaction was observed in a low number of neocortical, midbrain, and pontine neurons. The maximum number of positive neurons was detected in the cerebellar cortex (Purkinje cells) and hippocampus.

Key Words: *neurosteroids; brain neurons; 3β-hydroxysteroid dehydrogenase*

Neurosteroids are steroid hormones produced in the brain and other compartments of the nervous system. Published data suggest that they are synthesized in both neurons and glial cells. Their production was detected in cerebellar Purkinje cells, hippocampal neurons, and neocortex. It was found that during organogenesis of the brain they modulate neuronal development, dendrite growth, formation of spines, and can act as transmitters and modulators in synapses. Information of the synthesis and localization of neurosteroids was obtained by *in situ* hybridization and biochemical and immunocytochemical analysis [1,7-10]. We found no published data on light-optical histochemical detection of enzymes catalyzing their synthesis. However, this approach can supplement the current methods and can be useful for evaluation of the role of neurosteroids in brain development and aging and in the formation of pathological changes and reaction to various factors. This approach has some advantages. For instance, the use of various substrates allows detection of different enzymes, *e.g.* dehydrogenases catalyzing various stages of steroidogenesis [3]. An important aspect is the possibility of quantitative cytospectroscopic evaluation of the activity of dehydrogenases involved in the synthesis of steroid hormones. It should be noted that histochemistry of dehydrogenases is well-developed, easy, and cheap method.

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Here we studied the possibility of histochemical detection of 3β-hydroxysteroid dehydrogenase (HSD), the key enzyme of steroidogenesis catalyzing conversion of pregnenolone into progesterone [5] in neurons of some brain structures in rats.

MATERIALS AND METHODS

We studied activity of HSD in the brain of adult male rats weighing 130-150 g (n=10). The animals were decapitated, the brain was promptly removed, and medulla oblongata, pons, mesencephalon, cerebellum, diencephalon, and frontoparietal and parietal lobes were isolated as described elsewhere [2,6]. Each structure was embedded in a separate block for obtaining cryostat section (4-5 sections, 60 μ) on a Leica CM 1850 cryostat. The sections were mounted on coverslips and covered with an incubation solution prepared as described previously [3] and containing dehydroepiandrosterone (substrate), NAD, and NBT (all reagents were from Sigma). The reaction was carried out in a thermostat at 37°C for 30 min. The preparations were embedded in Canadian Balm; the slides were examined under an Olympus microscope.

RESULTS

Analysis of preparations revealed only low number of HSD-positive neurons in the studied brainstem structures, whereas most neurons were not visualized,

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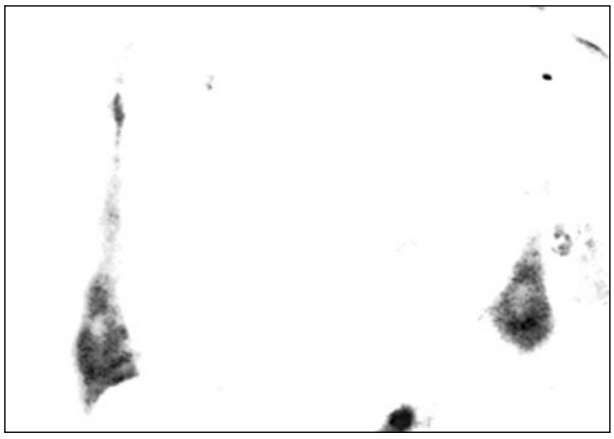

Fig. 1. Reaction for HSD in layer V pyramid neurons of the neocortex, ×400.

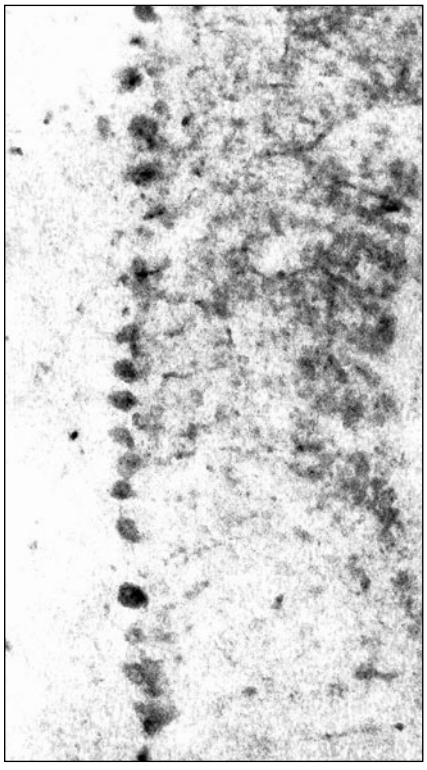

Fig. 2. Reaction for HSD in the cerebellar cortex. Intensive reaction in the bodies and dendrites of Purkinje cells (ganglionic and molecular layers). No reaction in the granular layer, ×400.

which suggests that steroid hormones are synthesized only in some nervous cells; moreover, the reaction products (violet formazan granules) in HSD-positive neurons were seen not only in the perikaryons, but also in cell processes.

In the frontoparietal and parietal lobes of brain cortex, the positive reaction was observed in only few V and VI layer neurons scattered at a distance of 100-200 μ and more from each other. The reaction products were seen in both the perikaryon and processes (dendrites and axons, in cases then the site of its orifice from the perikaryon appeared in the section plane; Fig. 1). On the contrary, in the hippocampus the positive reaction was detected in all, or at least in the majority of neurons. In the cerebellar cortex, the positive staining was seen in neuronal bodies and dendrites of the majority of ganglionic layer cells (Purkinje cells), while granular layer cells remained unstained (Fig. 2, 3). These findings agree with the data on intensive synthesis of neurosteroids in Purkinje cells [8-10]. In different brainstem regions, more or less numerous groups of neurons of some nuclei were found. Similarly as in other brain structures, the reaction products were seen in both perikaryon cytoplasm and processes (Fig. 4).

It should be taken into account that neurosteroids are synthesized in certain neurons and during certain periods, *i.e.* the number and topography of these cells and intensity of neurosteroid formation in them can vary [1,7,9,10]. This is confirmed by the effect of prenatal stress on activity of enzymes catalyzing the formation of neurosteroids [4]. These results suggest that cerebellar cortex and hippocampus are the most convenient structures for cytospectrometric assay of enzyme activity, because in these structures the posi-

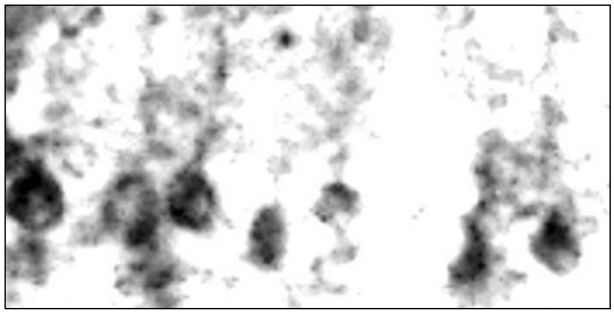

Fig. 3. Reaction for HSD in the bodies and dendrites of Purkinje cells. No reaction in axons, ×900.

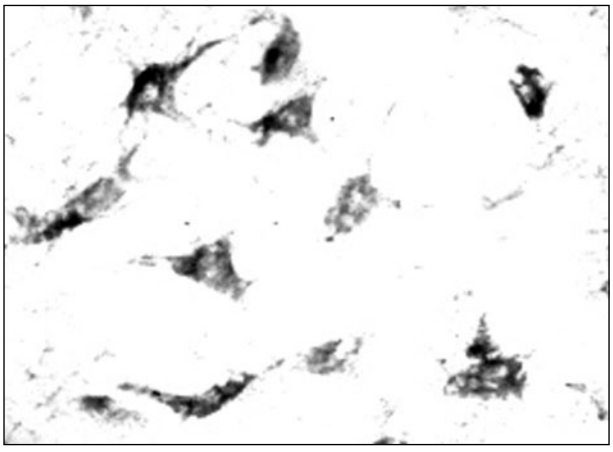

Fig. 4. Reaction for HSD in brainstem neurons (mesencephalon), ×400.

tive staining was detected in a great number of densely located neurons with well-studied functions.

Our results gave us grounds to conclude that histochemical study of dehydrogenases catalyzing different stages of steroid hormone synthesis can be a promising approach in studying of the role of these compounds in the development and functioning of various brain structures.

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