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FORMATION OF CATECHOLAMINERGIC STRUCTURES IN THE MEDIOBASAL HYPOTHALAMUS OF THE RAT

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Information on the formation of catecholaminergic (CAE) structures of the hypothalamus is very sparse and is confined mainly to the postnatal period of development. The fluorescence histochemical method of Falck and Hillarp can detect CAE neurons as early as the 2nd-3rd day of postnatal life [10, 12]. Progress achieved in recent years in immunocytochemistry has led to the discovery of neurons containing marker enzyme of catecholamine (CA) synthesis in the hypothalamus of 13- to 15-day fetuses [4, 13]. However, it has not provided the answer to the question whether these enzymes can catalyze CA formation in the prenatal period of development.

The aim of this investigation was to study the formation of CAE structures in the mediobasal hypothalamus (MBH) of rat fetuses by the more sensitive histofluorescence method with glyoxalic acid.

EXPERIMENTAL METHOD

CA in the hypothalamus of 16- and 18-day Wistar rat fetuses were determined by a fluorescence histochemical method using glyoxalic acid in the modification in [5]. To intensify the fluorescence, injection of pargyline (100 mg/kg), a monoamine oxidase inhibitor, was given to the pregnant rats 2 h before sacrifice. Control animals received an injection of reserpine (5 mg/kg). After decapitation, the brain was quickly removed, the MBH region isolated, and a fragment 1-3 μ thick was placed in a cryostat (−30°C). Serial frontal sections 10-25 μ thick were dried at room temperature on a slide and incubated for 1-3 min in a 1% solution of glyoxalic acid, made up in phosphate buffer (pH 7.4). The sections were dried with benzene (20-30 min) and kept in a drying cupboard at 80°C. They were then mounted in mineral oil and examined in the LYUMAM-IZ luminescence microscope.

EXPERIMENTAL RESULTS

On the 16th day of prenatal development, nuclear zones begin to be formed in MBH. At this stage small cells, with the characteristic green fluorescence of CA, were found along the 3rd ventricle. Terminal fields are found both in the periventricular and in the more lateral regions of MBH: axon terminals of varied caliber, mainly small and delicate (Fig.

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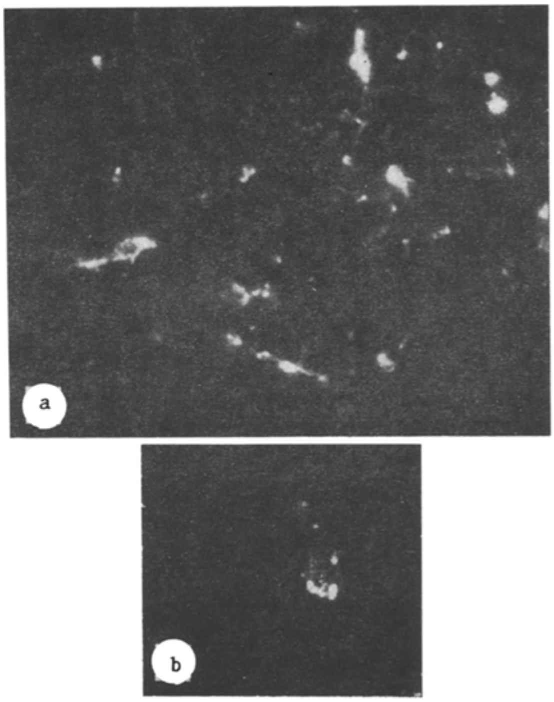

Fig. 1

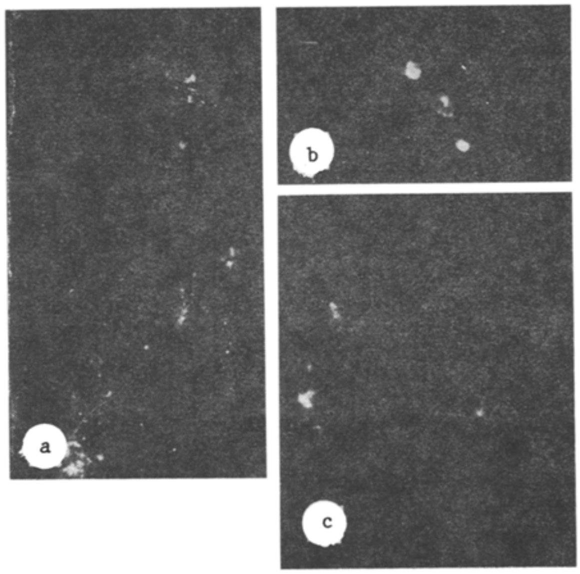

Fig. 2

Fig. 1. 16th Day of prenatal development. Periventricular region (1200x). a) CAE cells and terminals of varied caliber; b) cones of growth.

Fig. 2. 18th Day of prenatal development. Paraventricular nucleus (PVN) (1200×). a) Periventricular part of PVN – CAE cells with axons running along 3rd ventricle; b) magnocellular part of PVN – nonfluorescent cells surrounded by large CAE terminals; c) lateral part of PVN, changing into zona incerta.

1a). Sometimes processes of nerve cells, running toward the side of the ventricle, and with large, club-shaped, strongly fluorescent expansions at their distal end, evidently cones of growth, could be seen (Fig. 1b).

On the 18th day of prenatal development the following nuclei could be distinguished in MBH, in the rostrocaudal direction: paraventricular (PVN), arcuate (AN), ventromedial (VMN) and dorsomedial (DMN). During this period the number of cells with the green fluorescence characteristic of CA was considerably increased, as also was the intensity of fluorescence. In all the regions studied, CAE nerve fibers and terminals of varied caliber could be seen. Abundant fluorescence was found in PVN. At the most rostral level of MBH, in the periventricular part of PVN, there were fusiform fluorescent cells with processes running toward AN, parallel to the wall of the 3rd ventricle (Fig. 2a). In the magnocellular part of PVN a group of nonfluorescent cells surrounded by CAE terminals was found (Fig. 2b). The character of the large nerve terminals with intensive fluorescence suggests that they are terminals of noradrenergic neurons of the locus coeruleus [10]. In the most lateral part of PVN, changing without any visible boundary into the zona incerta, a fairly large group of small, round CAE cells with horizontal varicose processes was found. Many small terminals interwove here with very thin fibers entering from the lateral side from the perifornical region. Large, strongly fluorescent terminals also were quite numerous (Fig. 2c).

At the rostral level of MBH the anterior part of VMN could be seen. On its dorsolateral border there were small fusiform, fluorescent cells, usually bipolar. Their processes, intersecting with CAE fibers entering the nucleus from the lateral and ventrolateral side, created a complex pattern of interweaving. On the boundary of the nucleus there were nerve terminals of varied caliber ranging from small to very large (Fig. 3a). At the middle level, VMN became ovate in shape and consisted of anterior, middle, and lateral parts. Over the whole extent of the nucleus the number of CAE cells was small. Individual cells with weak and average intensity of fluorescence were located on the ventral border of the nucleus. Some of them sent axons toward AN (Fig. 3b).

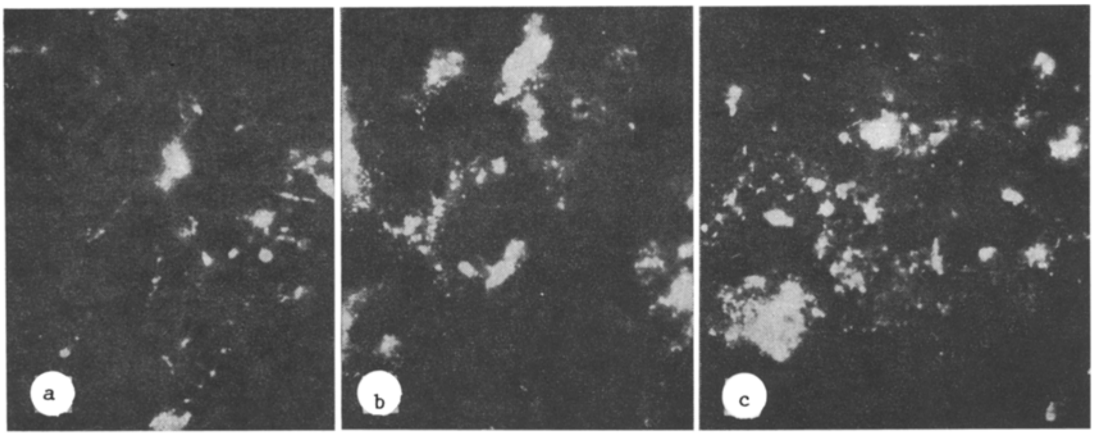

Fig. 3. 18th Day of prenatal development (1200×). a) Rostral part of VMN; b) middle part of VMN – CAE neurons on ventral boundary of nucleus; c) CAE neurons of arcuate nucleus with processes ending in an arch toward the median eminence.

A group of small CAE cells with processes running toward the median eminence could be seen in AN, especially in its ventral part (Fig. 3c). Single strongly fluorescent cells were found in the periventricular part of DMN. More caudally in DMN and a little dorsally to it there were small CAE cells and a fairly dense network of small terminals. Laterally to DMN, in the perifornical region, there were large cells with a weak and average intensity of fluorescence.

The results are evidence that the formation of CAE structures of MBH in rats begins in the period of embryonic development. In the 16-day-old fetus single cells able to synthesize and store catecholamines were already observed. By the 18th day of development the number of CAE cells in MBH was considerably increased and the topography of their distribution corresponded to that in the adult animal. Neurons of the parvocellular nuclei of MBH, namely PVN, AN, VMN, and DMN, are formed on the 15th-18th days of prenatal development [2, 6]. It can therefore be tentatively suggested that the beginning of CA synthesis in the hypothalamus coincides with the end of mitotic division and emergence of the neurons into differentiation, or takes place soon after that. A similar pattern of development also has been found for neurons of the medulla and mesencephalon [8, 11]. By the use of antiserum to tyrosine hydroxylase, and by an immunohistochemical method, groups of immunoreactive cells corresponding in their topography to the CAE neurons are found for the first time on the 15th-16th days of prenatal development [4, 13]. Specht [13] suggests that 1 or 2 days after the appearance of tyrosine hydroxylase in the cell, the enzyme already possesses catalytic activity. High tyrosine hydroxylase activity has been found in extracts of the forebrain between the 15th and 17th days of prenatal development [3].

Terminal fields and infrequent, very thin CAE fibers, sometimes with cones of growth, were observed in the 16-day fetuses. Judging from the caliber of the terminals, most of them are evidently endings of intrahypothalamic dopaminergic neurons. However, individual large terminals found in MBH during this period may be endings of noradrenergic neurons in the medulla. The first terminal fields are found immunohistochemically in the hypothalamus in 15-day fetuses. At this time noradrenergic pathways running into the hypothalamus from the medulla, and a nigrostriatal pathway from the mesencephalon are formed [13]. Five nerve bundles, arising in neurons of the medulla and mesencephalon, are found in 18-day fetuses. All run in the composition of the medial forebrain bundle to the lateral hypothalamus, where some of the fibers turn toward MBH [14]. The character of distribution of the terminals and nerve fibers, which we observed, indicates that many of them do in fact reach MBH.

The large number of nervous processes with cones of growth observed in 18-day fetuses may indicate their active growth (lengthening and branching), and also the formation of synaptic contacts. The presence of specialized receptors on the membrane of cones of growth is known to enable them to recognize target cells, with which the cones of growth form the first synaptic contacts [7]. Synapse-like structures were found electron-microscopically in AN in 17-day fetuses, and the first synapses, although still immature, were found in 18-day fetuses, most frequently on primitive dendrites [7].

Thus by the 18th day of prenatal development of MBH it has a CAE system of quite complex organization, which is already capable of securing the participation of catecholamines in regulation of the trophic functions of the pituitary. Evidence that catecholamines may be involved in the control of the hypothalamic-pituitary-gonads system is given by the results of physiological investigations: on intracerebral injection of 6-hydroxydopamine in 16-day-old fetuses, leading to selective degeneration of noradrenergic terminals, the rise of the testosterone level characteristic of 18-day-old fetuses does not take place [9].

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ULTRASTRUCTURAL MANIFESTATIONS OF FUNCTIONAL PLASTICITY OF THE ENDOTHELIUM OF THE RENAL GLOMERULI IN VERTEBRATES

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KEY WORDS: endothelium; renal glomeruli; ultrastructure; phylogeny.

The kidney is one of the first biological objects to have undergone thorough submicroscopic investigation in the very earliest stages of creation and development of electron microscopy [5, 7, 8, 10, 11]. In accordance with an empirical rule formulated by Ugolev [4], if a feature discovered in organisms occupying extreme position in the phylogenetic series is constant or varies within certain limits, this feature in intermediate species will correspond to the general rules for that series. The kidney as an organ appears and develops only within the vertebrates, and evolution of renal function has been examined only along the path from fish to mammal [1].

Accordingly, it was decided to undertake a comparative morphological study of the fine structure of the endothelium of the renal glomeruli in fishes and man.

EXPERIMENTAL METHOD

Pieces of tissue from an intact zone of the kidney in patients with Wilms' tumor aged 2, 4, and 6 years (altogether four observations) were studied. The ultrastructural compo-

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