Neurosecretory Cells of the Amygdaloid Complex during Estrous Cycle

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Ultrastructure of neurosecretory cells of the dorsomedial nucleus of the cerebral amygdaloid complex (one of the main zones of sexual dimorphism) was studied in different phases of the estrous cycle. The characteristics of the "light" and "dark" cells change depending on the concentrations of sex steroids during estrus and metestrus.

Key Words: *neurosecretory cells; sexual dimorphism zones; cerebral amygdaloid complex; sex steroids*

In our previous study we described neurosecretory cells (NC), first detected by us in zones of sexual dimorphism (dorsomedial and anterior cortical nuclei of the cerebral amygdaloid complex, AC) [2]. Studies of histophysiology of AC dorsomedial nucleus showed that karyovolumetric parameters of its neurons carrying sex steroid receptors changed during the estrous cycle [1].

Here we characterized the ultrastructural features of NC in the AC dorsomedial nucleus during estrus and metestrus.

MATERIALS AND METHODS

The study was carried out on adult female Wistar rats (200-250 g) with regular estrous cycle. The animals were kept under optimal conditions (day/night regimen, ambient temperature, use of male pheromones). The stages of the estrous cycle were determined by cytological picture of vaginal smears. The animals were decapitated at the estrus and metestrus stages. The material for electron microscopy was collected under an MBS-9 glass, fixed with 2.5% glutaraldehyde in phosphate buffer (pH 7.4), and postfixed in 2% OsO_4. The sections were made on an LKB III ultratome, contrasted with lead citrate [14], and examined under a JEM 200 EX electron microscope (75 kV).

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RESULTS

Analysis of NC characteristics at the estrus and metestrus stages showed not only "light" (clear nucleus and clear cytoplasm) and "dark" cells (dark nucleus and dark cytoplasm, intensely absorbing electrons), but also transitional forms (clear nucleus and dark cytoplasm). The most part of NC belonged to this latter category, which necessitated their differentiation by ultrastructural characteristics of the cell nucleus components and cytoplasmic organelles.

Considering the NC ultrastructure reflecting their functional activity, we came to a conclusion that the status of some NC (about 21%) could be defined as "moderate activity". It was characterized by the appearance of signs of transcription activity in the nuclei: perichromatin fibrils, focal accumulations of perichromatin and interchromatin granules. The nucleus remained clear, while the cytoplasm became moderately electron-dense because of increased number of granular cytoplasmic reticulum tubules, attached and free ribosomes, polysomes, hypertrophic dark mitochondria, moderately hypertrophic Golgi complex with dilated lumens of the cisterns, increased number of transport vesicles, appearance of small and large osmiophilic secretory granules, and increased number of lysosomes. This determined cytoplasm osmiophilia, which became dark against the background of light neuropil and clear cell nucleus.

The ultrastructure of most NC (about 50%) during the estrus phase reflected the state of "increased functional activity". Abundant granular material was seen in the karyoplasm and enlarged nucleolus. The dark granular component of the nucleoli disappeared from its surface and formed accumulations. Perichromatin fibrils were seen in areas of loosely disposed interchromatin granules. The perinuclear space was dilated, nuclear pores increased. The tubules of the cytoplasmic reticulum were dilated and transformed into cisterns. The number of attached ribosomes decreased and that of free ribosomes and polysomes increased. The number of hypertrophic dark mitochondria increased in all zones of the cell. The Golgi complex was hypertrophic. Some its complexes with dilated cisterns, well-developed vacuolar component, and numerous transport vesicles were seen. Elementary neurosecretory granules were seen near the Golgi complex or near the plasma membrane at the cell periphery. The cytoplasm of these neurons was more electron dense and more osmiophilic compared to cell nucleus and neuropil.

The percentage of "dark" electron-dense cells was about 26% at the estrus stage. High electron density of the enlarged nucleus was due to the presence of abundant granular material (clear and dark), osmiophilic powder-like substance, and sites of heterochromatin. The nucleolus in such cells could be loose, enlarged, with well discernible granular and fibrillar component, or shrunk and compact. Presumably, it is possible to differentiate between NC variants at this stage, but this problem requires further investigation. Sharply dilated cisterns of the cytoplasmic reticulum were seen in the perikaryon of "dark" cells, with accumulations of polysomes and free ribosomes between the cisterns. The Golgi complex was well developed: 3-4 complexes were discernible, characterized by a well-developed membrane component; formation of secretory granules was seen nearby. Many hypertrophic mitochondria were seen, some of them light. Because of high osmiophilia of the cytoplasm, lysosomes and elementary neurosecretory granules located (in small amounts) in different zones of the cytoplasm remained unseen.

During metestrus 12% NC were in a state of moderate activity, 25% in a state of high activity, and 60% were dark.

Few NC, whose functional condition could be defined as "resting", were detected at the estrus and metestrus stages. These cells had clear euchromatin-rich nucleus with compact nucleolus in the central zones and small accumulations of heterochromatin. Narrow tubules of the granular cytoplasmic reticulum, moderate number of dark mitochondria, Golgi complex, primary lysosomes, small accumulations of polysomes, and separate elementary neurosecretory granules (50-200 nm in diameter) were seen in the cytoplasm.

We know that the levels of estradiol and luteinizing hormone (LH) are low during the metestrus, the peak of estrogens is recorded at 12 h of proestrus, and of LH at 18 h of the same stage of the estrous cycle [3]. LH release in the presence of increasing estrogen concentration leads to ovulation, which occurs during the estrus.

It seems that some NC either were either insensitive to fluctuations in sex steroid levels (because of temporary desensitization) or reacts asynchronously. This latter hypothesis is based on the data on heterogeneity of estrogen receptors: two types of estrogen receptors (α and β) are distinguished [12]. It was shown that their biological roles are different: by forming the hormone-receptor complexes, subtype α receptors activate transcription processes in the cell, while subtype β receptors inhibit these processes [5,11].

The differences between the "dark" and "light" cells can be explained by the mechanism of sex steroid effects on the cell genome [6]. It is known that these hormones easily penetrate into the cell and bind to specific receptors in the cytoplasm, forming hormone-receptor complexes. Nuclear translocation of these complexes and their binding to the acceptor sites of chromatin leads to genome activation. Changes in the status of the nuclear matrix are also possible, as RNA biosynthesis is directly related to it [10] and rDNA amplification, explaining the rapid increment of ribosomes during the metestrus, is a reality [4]. All these processes ensures intensive protein synthesis in the cell, which is needed for the creation of a necessary pool of cytosol receptors presented by hydrophilic proteins and other substances (arginine-vasopressin, enkephalin, substance P, catecholamines) [7,8,11,13, 15]. It seems that gene activation starts during the estrus stage under the effect of peak concentrations of estradiol and LH [9], the expression of these genes during the metestrus stage manifests in increased activity of the protein-producing system of the cell.

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