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INFLUENCE OF ESTRADIOL ON NEURONS OF NUCLEUS BASOMEDIALIS IN THE AMYGDALOID COMPLEX OF FEMALE RATS

DIJANA DJELIĆ*, OLIVERA LOZANČE*. N. DJELIĆ** and D. DREKIĆ*

*Department of Anatomy and **Department of Biology, Faculty of Veterinary Medicine, University of Belgrade, Bul. JNA, 18, 11000 Belgrade, Yugoslavia

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The amygdaloid complex (AC) of rat brain is sensitive to sex steroids, especially during neonatal development. The aim of this investigation was to determine possible changes in neuron morphology of the basomedial nucleus (BMN) of the AC in female rats treated with estradiol. Two straining techniques were used: (1) standard histological staining with hematoxylin-eosin and (2) the Golgi method. Three types of neurons: pyramidal, fusiform and stellate were subjected to stereologic analaysis. Estradiol significantly increased the volume of neuronal soma of all three neuronal types at 16 and 90 days of age, whereas at day 38 changes were observed only in fusiform and stellate neurons. In addition, the volume of cell nuclei was increased but the number of neurons per unit area decreased at all experimental intervals examined. Therefore, estradiol causes stereologically detectable changes in the size and number of neurons in BMN AC.

Key words: estradiol, basomedial nucleus, amygdaloid complex.

INTRODUCTION

Estrogens exert their biological effects mainly via specific intracellular receptors. Immunohistochemical studies indicate that estrogen receptors in the unbound state are predominantly located in the cell nucleus (Greene and Press, 1986; Warembourg et al., 1998; Joanne and Blaustein, 1999). Binding od estradiol results in activation of the receptor (Muller et al., 1983). The transformed form of the receptor is a homodimer that readily binds to specific DNA sequences designated as ERE (estrogen response elements; Berg, 1989).

The effects of estradiol have been studied in many cell types, under *in vitro* and *in vivo* conditions. Subcellular events which have been noticed include an increase in thymidine incorporation and cell growth, increased progesterone

receptor synthesis (Berthois et al., 1986), progression through the S phase of the cell cycle (Wilding et al., 1988), enhanced expression of protooncogenes *c-ras, c-fos* and *c-myc* (Murayama et al., 1988).

Having in mind the well established fact that most cells in the mammalian body contain steroid receptors, it is not surprising that steroid hormones can influence various biochemical processes in the brain. It has been shown that estrogen stimulates dopamine release and neuronal excitability in the striatum (Becker and Ramirez, 1981) and alters the levels of synthetic or degradative enzyme systems for amino acid neurotransmitters in extrahypothalamic areas (Maggi and Peeres, 1985). In addition, estrogen causes global activational effects on sensomotor function in the rat and humans, al well as enhancing seizure activity (Mattson and Cramer, 1985) and spontaneous firing of neurons in the male rat cerebral cortex (Kelly et al., 1977).

The limbic system contains target neurons responsive to sex steroids (Mizukami et al., 1983; Philis and O'Regan, 1988). The amygdaloid nuclear complex (AC) in the limbic system is capable of concentrating sex steroids (Bless et al., 1977; Warembourg et al., 1998; Stomati et al., 1998). The most prominent effects of gonadal steroids on AC can be observed during neonatal development (Rories and Spelsberg, 1989).

The aim of this experiment was to evaluate possible morphometric changes in neurons in the basomedial nucleus of AC in neonatally estrogen-treated female rats.

MATERIALS AND METHODS

Animals. Female Wistar rats were kept under standard conditions (adequate room temperature, illumination, food and water *ad libitum*). For each experimental point, 10 control and 10 treated females were used.

Treatment. The animals were treated (i.p.) with a single dose (1mg per animal) of estradiol-dipropionate at 3 days of age and subsecquently sacrificed on day 16, 38 of 90 or postnatal life.

Histological procedure. The animals were sacrificed under ether narcosis and the brain was removed immediately from the skull, the amygdaloid complex was isolated and prepared for staining either with hematoxylin and eosin (HE), or by Golgi the method (Drekić and Malobabić, 1987) as follows.

Staining with hemastoxylin-eosin (HE). The amygdaloid complex (AC) was fixed in Bouin solution. After embedding in paraffin serital sections (5-6 mm thick) were cut and stained with HE staining the following morphometric parameters of the basomedial nucleus were analyzed: the volume of neuronal nuclei (Vn) (μm^3) and number of neurons per unit area (N_A) (mm^2).

Staining by the Golgi method. After four weeks of fixation in 10% neutral formaildehyde, the brains were divided into smaller biocks which were impregnated by a modification of the Golgi method (Drekić and Malobabić, 1987). Microscopic slides stained by this method were used for analysis of the volume of neuronal soma (V_s) (μm^3).

Stereological analysis. Using a light microscope, the volume of neuronal nuclei (Vn) (μm^3) and volume of neuron soma (Vs) were determined using the formula for a rotatory ellipsoid conus and the number of neurons per unit area (N_A) (mm^2) using Weibes's multipurpose test system (P:42). Neurons were classified according to de Olmos (1990). Micrographs were taken on an NU_2 Carl Zeiss microscope. (Jena). The statistical significance of the differences between the parameters measured in treated and control animals was determined using Student's *t*-test.

RESULTS

Three types of neurons (pyramidal, fusiform and stellate) were observed on the microscopic slides stained with hematoxylin-eosin, as well as those stained by the Golgi method (Drekić and Malobabić, 1987).

Histologic analysis of BMN revealed the existence of two subregions: anterior (dorsomedial) and posterior (ventromedial). The determination of these regions is possible on the basis of differences in topographic location, their size, neuron body shape, and density of their populations. The most dominant types of neurons in the dorsomedial subregion were pyramidal and fusiform neurons, whereas in the ventromedial part fusiform and stellate neurons comprised the vast majority of the cell population.

Pyramidal neurons exhibited larger diameters of nuclei and soma in comparison to fusiform and stellate neurons.

Changes in the volume of neuronal soma (Vs) (μm^3) are presented in Table 1. In estrogen treated female rats, the volume of neuronal soma of all three types of neurons was increased ($p<0.001$) on the 16th and 90th day of postnatal life, whereas at day 38 the increase was observed only in fusiform ($p<0.05$) and stellate neeurons ($p<0.01$).

Table 1. Stereological measurements of the volume of neuronal soma (Vs) (μm^3) of BMN AC in control and oestrogen-treated female rats;
*, p< 0.05; **. p<0.01; ***.P<0.001.

cell type	control Vs (μm^3)	treated Vs (μm^3)	control Vs (μm^3)	treated Vs (μm^3)	control Vs (μm^3)	treated Vs (μm^3)
pyramidal	2571.08	4545.18***	3595.14	3651.65	3388.09	3981.49***
fusiform	2441.20	3715.33***	2649.10	2961.25*	2914.88	3562.19***
stellate	1957.08	2935.27***	2231.76	2647.09**	2672.37	3293.46***
	16th day of life		38th day of life		90th day of life	

After estrogen treatment, the volume of the cell nuclei (Vn) (μm^3) was increased ($p<0.001$) on the 16th, 38th and 90th days of age, compared to the respective controls. (Table 2). In contrast, the number of neurons per unit area (N_A) (mm^2) was significantly ($p<0.001$) decreased for all neuron types (Table 3).

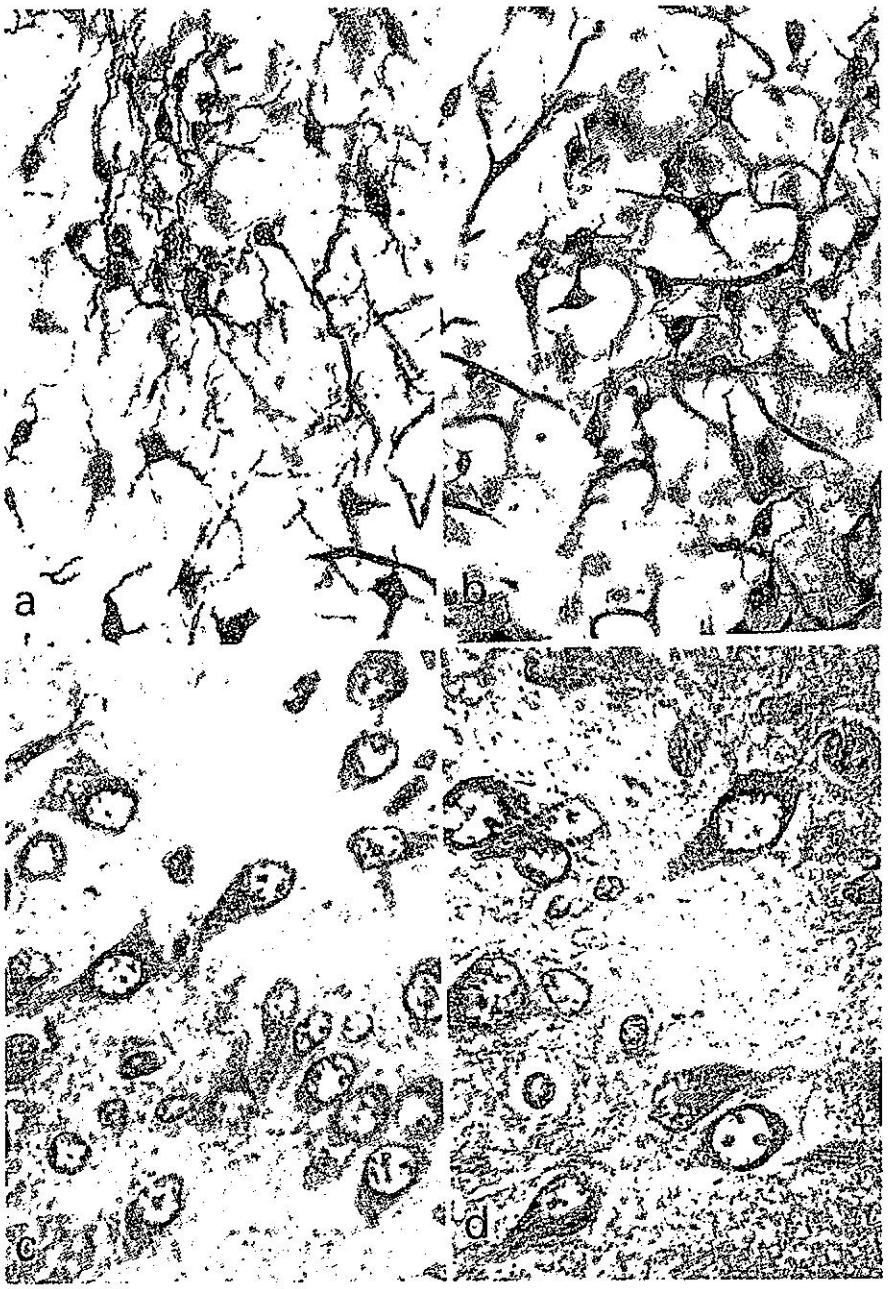

Figure 1. Basomedial nucleus of amygdaloid complex (BMN AC) in 16-day-old female rats. a) control, b) treated (Golgi method, x 544); and c) control, d) treated (HE x1080).

Table 2. Stereological measurements of cell nuclei volume (Vn) (μm^3) BMN AC in control and oestrogen - treated female rats.
*.p<0.05; **.p<0.01; ***.p<0.001.

cell type	control Vn (μm^3)	treated Vn (μm^3)	control Vn (μm^3)	treated Vn (μm^3)	control Vn (μm^3)	treated Vn (μm^3)
pyramidak	753.76	830.68***	443.40	506.42***	333.99	348.70***
fusiform	428.66	498.75***	296.69	350.19***	182.74	251.97***
stellate	294.16	357.01***	210.51	251.18***	127.49	178.49***
	16th day of life		38th day of life		90th day of life	

Table 3. Stereological measurements of BMN AC in control and oestrogen-treated female rats expressed as the number of neurons per unit are (N_A) (mm^2).
*.p<0.05; **.p<0.01,; ***.p<0.001.

cell type	control V_A (mm^2)	treated V_A (mm^2)	control V_A (mm^2)	treated V_A (mm^2)	control V_A (mm^2)	treated V_A (mm^2)
pyramidak	344.37	305.19***	262.57	216.52***	114.10	103.79***
fusiform	283.20	253.95***	240.58	207.58***	144.34	126.47***
stellate	273.57	240.58***	228.21	204.73***	155.34	137.47***
	16th day of life		38th day of life		90th day of life	

It is evident that estrogen caused streologically detectable changes in size and number of neurons in the BMN of the amygdaloid complex in neonatally treated female rats.

DISCUSSION

Estrogen hormones exert a strong influence on sensitive brain regions followed by physiological changes such as excitatory or sedative effects (Phillis and O'Regan, 1988; Hutchinson et al., 1995; Ostterlund et al., 1998). In addition, it has been shown that estrogens strongly influence certain brain regions during a critical period of neonatal development, including the amygdaloid complex (Phillis and O'Regan, 1988; Cvetković 1992; Gray and Bingaman, 1996). Thus, it has been described that application of estrogens in the neonatal period causes a significant increase in the relative volume of cell nuclei in the amygdaloid complex of juvenile and prepubertal male rats (Pantić and Drekić, 1982). Moreover neonatal treatment with estrogen still increased the volume of cell nuclei in 6-munth-old animals, whereas in 12 - month-old animals no significant difference could be observed (Drekić et al., 1988).

Having these findings in mind we analyzed possible stereological changes in pyramidal, stellate and fusiform neurons of the basomedial nucleus in female rats exposed to estradiol.

All neuronal types responded to estradiol gavage on the 3rd day of postnatal life. The absolute volume of neurons, as well as well as nuclear diameter were increased, indicating activation of biochemical processes com-

patible with subcellular morphological changes. Namely, increase in cell nuclei size shold indicate relaxed chromatin conformation and an elevated level of transcription of estrogen-responsive genes (Alberts et al., 1994). In addition, since the total volume of examined cells was also increased, we assume that activation of estrogen-responsive genes is followed by an increase in protein synthesis in the cytoplasm. Therefore, complex interaction of activated estrogen receptors and chromatin probably causes long-lasting changes in chromatin conformation and increase in protein synthesis and other cytosolic processes that are stereologically expressed as enlargement of the cytoplasm.

Moreover, it should be pointed out that, besides all the above mentioned stereological changes, nucleoli in susceptible neurons were more prominent, or it was even possible to observe two nucleoli. These results are in accordance with previous findings (Vazquez-Nin et al., 1986) of cytological changes compatible with elevated protein synthesis level.

Our experimental data indicate that estradiol causes morphologic changes that reflect increased biochemical activity of target neurons. We assume that estrogen in neonatally treated rat females may have provoked changes that were observed subsequently, possibly due to overall changes in neuroendocrine regulation and hormonal status. Recent data suggest that gonadal hormone receptors in the amygdala are located adjacent to corticotropin-releasing factor (CRF)-expressing neurons and in amygdaloid neurons they are likely to participate in central autonomic and neuroendocrine circuitry (Gray and Bingaman, 1996). It is not completely clear what is the biological significance of the reactivity of certain neurons to gonadal steroids. Nevertheless, numerous experimental data reveal the existence of different reactivity of male and female brains both to endogenous, and to administered sex hormones (Yokosuka et al., 1997; Lozanče 1991, 1996). Furthermore, the available data suggest that estrogen can modulate behavior and functions mediated by the amygdala and hypothalamus via differentially regulated estrogen receptor subtypes (Osterlund et al., 1998). Hence, the developmental and genetic differences can direct sex-specific organization of neuronal subpopulations followed by specific physiological and behavioral characteristics of adult animals.

A c k n o w l e d g e m e n t

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UTICAJ ESTRADIOLA NA NEURONE NUKLEUSA BASOMEDIALISA AMIGDALOIDNOG KOMPLEKSA ŽENKI PACOVA

DIJANA DJELIĆ*, OLIVERA LOZANČE*, N. DJELIĆ** i D. DREKIĆ*

SADRŽAJ

Amigdaloidni kompleks (AK) mozga pacova senzitivan je na polne steroide, naročito tokom neonatalnog razvića. Cilj ovog istraživanja bio je da se odrede moguće promene morfologije neurona bazomedialnog nukleusa (BMN) AK kod ženki pacova tretiranih estradiolom trećeg dana života. Pri tome su korišćene dve tehnike bojenja (1) standardno histološko bojenje hematoksilin-eozinom i (2) Goldži metoda. Stereološke analize obuhvatile su tri tipa neurona: piramidalne, fuziformne i stelatne. Zapažen je značajan uticaj estradiola na povećanje some sva tri tipa neurona kod životinja žrtvovanih 16. i 90. dana života, dok su 38. dana promene konstatovane samo kod fuziformnih i stelatnih neurona. Pored toga, došlo je do povećanja volumena ćelijskog jedra, kao i smanjenja broja neurona na jedinici površine kod svih tipova neurona. Prema tome, estraiol dovodi do stereološki detektabilnih promena u veličini i broju neurona po jedinici površine u BMN AK.