Acta Veterinaria (Beograd), Vol. 54, No. 5-6, 347-356, 2004.

UDK 619:612.018.2:632.693.2

INFLUENCE OF LHRH ON SEX HORMONE RECEPTORS IN THE AMYGDALA OF THE MALE RAT

DREKIĆ D, MRVIĆ-JOVIČIĆ VERICA, ZORIĆ Z, DJELIĆ DIJANA, BLAGOJEVIĆ ZDENKA and NIKOLIĆ ZORA

Faculty of Veterinary Medicine, Belgrade

(Received 23. June 2004)

Autoradiography was used to localize estrogen-accumulating cells in the amygdala (AMY), of male rats. with LHRH hormones of seven adult male rats (86 days old). Seven mail rats were each treated with an injection of luteinizing hormone-releasing hormone (LHRH, 25 μg) at 83 days old and 3 days later with 250 μCi ^{3}H-estradiol (E_2).

A control group of male rats was also treated with 250 μCi ^{3}H-E_2, two hours before sacrifice. Both groups were sacrificed at 86 days old.

In the control group of male rats, the nuclei of the AMY with the highest density of estrogen binding (receptors) were nucleus medialis (NM), nucleus corticalis (NCO), nucleus centralis (NCE) and massa intercalata (MI) of pars corticomedialis of AMY. These nuclei belong to the phylogenetically older corticomedial part of the AMY. Light to moderate labeling was present in the phylogenetically younger nucleus basomedialis (NBM) and nucleus basolateralis (NBL). Weak labeling was present in nucleus lateralis anterior (NLA) and nucleus lateralis posterior (NLP) both from the phylogenetically younger basolateral part of the adult male rat AMY. This distribution of estrogen receptors could be related to the biologically more significant influence of estrogen on the regions of response divergence than on regions of sensory convergence of AMY. In the male rats treated with LHRH+ ^{3}H-E_2, we noticed different a distribution of estrogen receptors, in the different types nuclei of neurons (nucleus of AMY).

We observed a smaller number of estrogen receptors in the nucleus of pyramidal neurons, while in fusiform and stellate neurons, a similar number of receptors was present as in the control group for NM, NCO, and NCE. In massa intercalata we found a large numberof receptors in the nuclei of neurons, in the older pars AMY. In younger pars AMY, NBL and NLP, we noticed a significant decrease of receptors for estradiol in the nuclei of pyramidal and fusiform neurons.

Key words: estrogen receptors, amygdale, male rats, autoradiography, types of neurons

Acta Veterinaria (Beograd), Vol. 54, No. 5-6, 347-356, 2004.
Drekić D *et al*. Influence of LHRH on sex hormone receptors in the amygdala of the male rat

INTRODUCTION

Luteinizing hormone – releasing hormone (LHRH) is released in pulses and triggers the production of gonadotropins, which stimulate the growth and release of eggs by the ovary. This has been best illustrated by experiments in which the actions of the decapeptide have been blocked by immunoneutralization or receptor antagonist treatment, which invariably leads to cessation or reduction of gonadotropin secretion, disruption of gonadal function, and infertility (Kalra and Kalva, 1983).

These peptides (LHRH) are used for treatment of a variety of hormone-dependent diseases like prostate cancer and endometriosis. Unfortunately, the superagonists stimulate luteinizing hormone-releasing hormone (LHRH) production, which in turn causes a surge of testosterone and androgens resulting in a "clinical flare." Eventually, they downregulate receptors, block release of luteinizing hormone (LH) and follicle stimulating hormone (FSH), and also prevent synthesis/release of testosterone or estrogen from the gonads (D'Souza *et al*., 2004).

In the rat, administration of an LHRH antagonist during proestrus results in rapid and complete inhibition of ovulation, demonstrating the importance of this neuropeptide in reproductive function. As recently reviewed by Kalra and Kalva (1983), a complex series of mechanisms involving biogenic amines, neuropeptides and circulating sex steroids acting on multiple hypothalamic and perhaps extrahypothalamic nuclei, all participate in the regulation of reproductive function (Barnes and Sarkar, 1995). It is not the purpose of this paper to describe in detail all the mechanisms controlling reproduction in males, but rather to give a general view of the distribution of receptors for estrogen, in phylogenetical older and younger parts of AMY in male rats. Testosterone is aromatied under the influence of the enzyme aromatase (Narada *et al*., 1993; Tsuruo *et al*., 1995; Beyer *et al*., 1994; Dellovade *et al*., 1995), in estrogen in nucleus AMY and especially in NCF.

MATERIALS AND METHODS

The presumptive distribution of estrogen receptors in the AMY complex of adult male rats was determined using autoradiography. Seven adult male rats (86 days old) received an intraperitoneal injection of 250 μCi tritated estradiol [^{3}H- E_2] (Amersham, 90-115 Ci/mmol) in 10% ethanol saline. The second group, 83 days old, were treated with 25 μg LHRH, and three days later were also given 250 μCi ^{3}H-E_2. Two hours after injection of ^{3}H-E_2 the animals were killed by ether anesthesia.

The brains were removed, fixed in Bouin solution and processed for autoradiography after embedding in paraffin. The region of AMY was cut in serially to 5 μm thick transverse sections, which were covered with ILFORD L4 emulsion, and exposed for 5 months at 4^o C. After development with KODAK 19, the sections were counterstained with hematoxylin.

The cell was considered as labeled if the number of nuclear silver grains exceeded twice the silver grain count in adjacent extracellular spaces and cytoplasm. Comparing investigated amygdaloid nuclei, we classified them, according to intensity of labeling, as highly, moderately and poorly labeled. In order to carry out more accurate morphological analysis, sections adjacent to those analyzed autoradiographically were stained with hematoxylin and eosin. Glial cells were differentiated from neurons according to their light microscopic characteristics. Micrographs were taken on an NU_2, Carl Zeiss Microscope (Jena) at a magnification of 1024 times.

RESULTS

In the control group, radioactivity was concentrated in the cell nuclei and to a lesser extent in the cytoplasm of the neurons of the brains of male rats injected with estradiol. The intensity of nuclear labeling varied among individual neurons and between neuronal populations of AMY.

The highest degree of labeling was present in the nuclei of the phylogenetically older, corticomedial part of amygdala: in NM, followed by NCE, NCO and MI respectively.

In the phylogenetically younger, basolateral part of AMY, numerous labeled cells (moderate labeling) were also observed in NBM and NBL.

A few scattered weakly labeled cells were present in the NLA and NLP of the phylogenetically younger, basolateral part of AMY, and in NCO in the phylogenetically older part of AMY. In male rats treated with LHRH, at the time of sexual maturity, at 63 days old, and then with labelled estrogen 2 hours before sacrifice, we found a different distribution of estrogen receptors in nuclei of neurons, in phylogenetically older parts of AMY.

For example, in NM NCO and NCE pyramidal neurons, had labelled receptors next to the nucleas membrane, as in nucleolus, which were eccentrically arranged.

In fusiform and stellate neurons, estrogen receptors were concentrated in the nucleolus.

However, in MI, we found a significantly increased number of receptors in nuclei of neurons, as was seen in the controll animals (Fig. 1).

The phylogenetically younger, basolateral part of AMY, NBL and NLP, which had a decreased number of receptors in control rats, under the influence of LHRH exhibited an even more decreased number of labelled receptors for estrogen (Drekić *et al*., 1995a, 1995b; Malobabic *et al*., 2002), especially in the pyramidal neurons. In fusiform and stellate neurons, we found them arranged in the nuclei of the neurons (Fig. 2).

We believe that LHRH influences the distribution sex hormone receptore in some neuron nuclei, in phylogenetically different parts of AMY as in other parts of the cortex.

Acta Veterinaria (Beograd), Vol. 54, No. 5-6, 347-356, 2004.

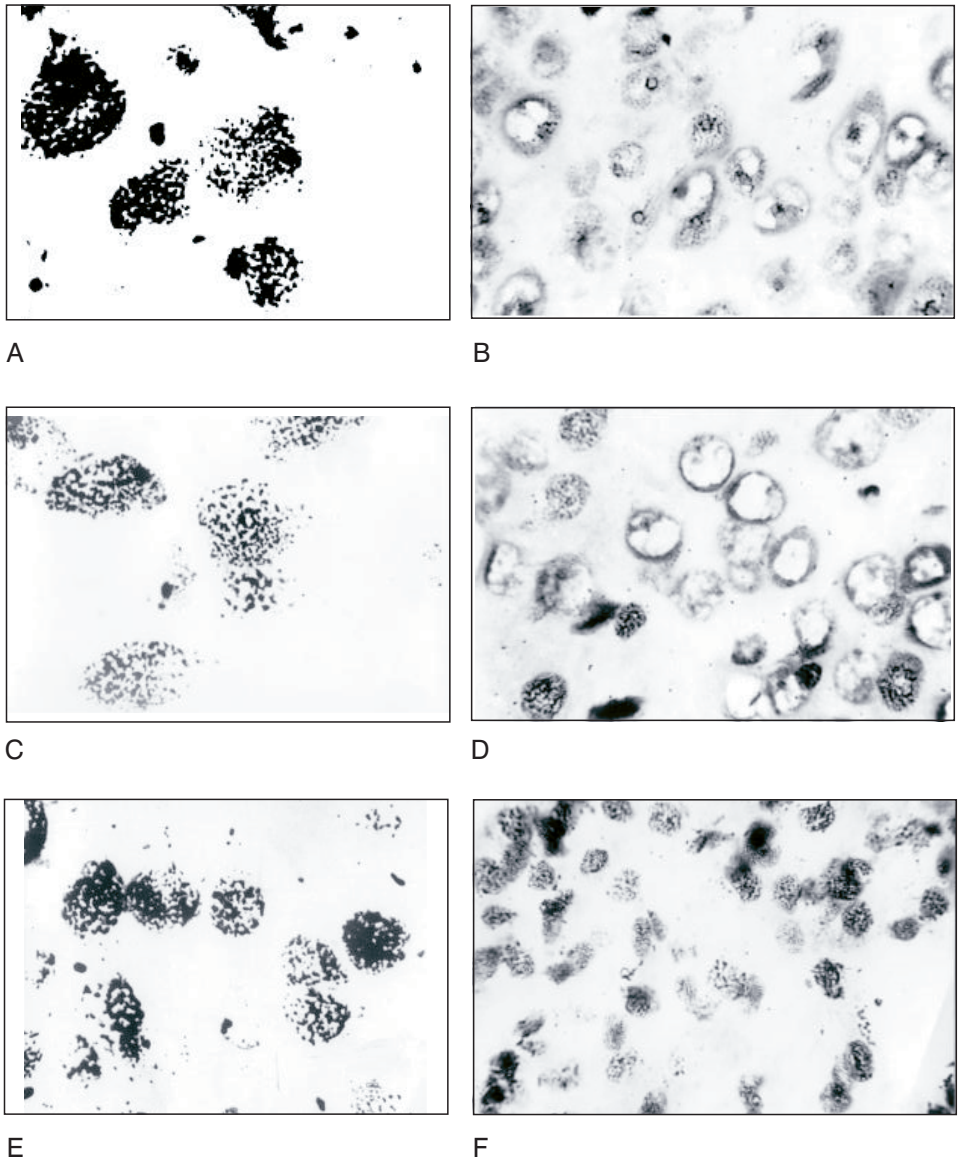

Figure 1. Labeled neurons in AMY of adult male rats (86 days old) treated with tritiated estrogen 2 hours before sacrifice (control group) A - *nucleus medialis*; C - *nucleus corticalis*; E - massa intercalata, or treated at 83 days old, with 25 μg LHRH and than at 86 days old, two hours before sacrifice with 250 μCi ^{3}H- E_2. B - *nucleus medialis*; D - *nucleus corticalis*; F - *massa intercalata*. Hematoxylin; X 1024

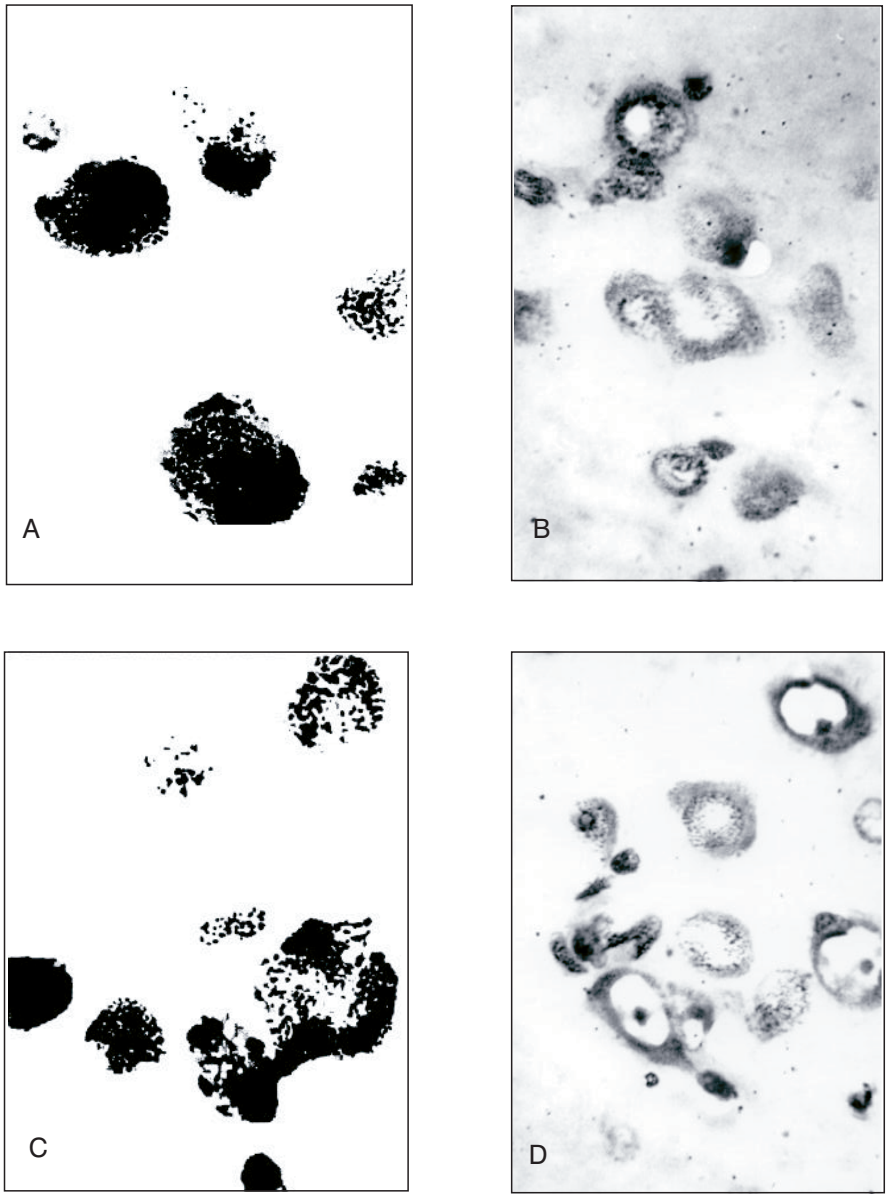

Figure 2. Labeled neurons in AMY of adult male rats (86 days old) treated with tritiated estrogen 2 hours before sacrifice (control group), A - *nucleus basolateralis* and C - *nucleus lateralis posterior;* or treated with 25 μg LHRH 83. days old, and then at 86 days old, two hours before sacrifice, with 250 μCi ^{3} H- E_2. B - *nucleus basolateralis* and D - *nucleus lateralis posterior.* Hematoxylin; X 1024

DISCUSSION

During this work, we studied the influence of LHRH on estrogen receptor distribution in some types of neurons. Male rats which had passed through puberty were used, as the hypothalamus and hypophysis is already formed. We know that, during embryonic development, LHRH neurons migrate from the medial olfactory placode of the developing nose through the nasal septum (Witkin, 1999; Susan M, 1998, and Terasawa *et al*., 1999) and into the forebrain with the nervus terminalis, arching into the septal-preoptic area and hypothalamus (Terasawa *et al*., 1999). Moore and colleagues (Moore and Wray, 2000) used in situ hybridization histochemistry and immunocytochemistry to study the prenatal expression of LHRH cells in the mouse and showed that postnatal LHRH neurons were "birth-dated" shortly after differentiation of the olfactory placode and before LHRH mRNA is expressed in cells of the olfactory pit (Terasawa, 1999) .

This elegant study therefore supported the hypothesis that all LHRH cells in the central nervous system (CNS) arise from a discrete group of progenitor cells in the olfactory placode, and that a subpopulation of these cells migrates into forebrain areas.

This species-dependent LHRH-neuronal organization might certainly contribute to the difficulty of pin-pointing mechanisms underlying the inhibitory or stimulatory actions of various factors on the reproductive system in various mammalian species.

The LHRH neuronal system differs from other types of neuropeptidergic systems, such as the neuroendocrine corticotropin-releasing factor system (CRF), which is essentially located in the parvocellular division of the paraventricular nucleus (PVN) of the hypothalamus under a compact cluster of perikarya forming a clear and defined nucleus.

The LHRH cell bodies are scattered and are distributed from the medial septum (MS)-diagonal band of Broca (DBB) to the caudal-medial preoptic area (MPOA) and ventral-rostral anterior hypothalamus within the rodent brain (Barnes and Sarkar, 1995), whereas another group of LHRH perikarya can be found in the ARC/ME of monkey and human brains.

There was no evidence of an elevation of testosterone, which occurs with the LHRH superagonists (D'Souza *et al*., 2004).

We and others have identified luteinizing hormone-releasing hormone (LHRH) in cells of the immune system in both animals and humans. LHRH is immunostimulant, and testosterone is an immunosuppressant. Because testosterone is known to modulate the concentrations of hypothalamic LHRH (Barnes *et al.*,1995), we wondered whether testosterone might also alter the concentrations of rat thymic LHRH. Two weeks after castration or sham castration, adult male rats were implanted with either vehicle or testosterone capsules. All animals were killed 4 days after capsule implantation. Thymic LHRH concentration increased significantly in castrated animals. Testosterone replacement prevented this increase. The concentration of the LHRH precursor, proLHRH, decreased significantly, but testosterone replacement prevented this

decrease. Steady-state concentrations of LHRH mRNA were not changed by castration or by hormonal replacement. In contrast to the post-castration increase in thymic LHRH, LHRH content of the hypothalamus decreased significantly. Whereas concentrations of LHRH were lower in the thymus than in the hypothalamus, proLHRH concentrations were much greater in the thymus. These data suggest that gonadal manipulation modulates LHRH molecular processing and its tissue concentration in the thymus in addition to those in the hypothalamus, and that the regulation of LHRH molecular processing by testosterone in the hypothalamus is different from that in the thymus. (Azad *et al*., 1998).

Our opinion is that LHRH affects the distribution of sex hormone receptors in AMY neurons.

While this latter group of LHRH cells is essentially neuroendocrine (i.e., neurons projecting to the infundibular system and directly involved in the control of gonadotropin release from the adenohypophysis), the more rostrally distributed LHRH neurons send their projections to various regions of the brain, including the ME (neuroendocrine), the interpeduncular nucleus, the medial amygdala, the mammillary complex and up to the periaqueductal region (Labrie *et al*., 1990).

In rats, a large concentration (the richest) of LHRH-immunoreactive and mRNA expressing neurons are localized within the MPOA, forming an inverted "V" surrounding the organum vasculosum of the lamina terminalis (OVLT) (Labrie *et al*., 1990).

It might, however, be too simplistic to believe that the MPOA is responsible in itself for the preovulatory LH surge and ovulation, because this hypothalamic structure receives massive input from numerous areas that may play a crucial role in orchestrating the afferent pathways driven by the physiological clock of ovulation and steroid production. (Barnes and Sarkar, 1995).

To test the hypothesis that the prepubertal increase in LHRH release is withheld by a dominant inhibitory neuronal system, Mitsushima *et al*. (1994), examined the role of g-aminobutyric acid (GABA; a recognized inhibitory neurotransmitter) (Andrea C. Gore *et al*., 1999), in the control of LHRH release before the onset of puberty. Their data provided evidence of a powerful GABAergic inhibition of the LHRH neurosecretory system in the prepubertal period, and this tonic inhibition may be a key factor in sexual maturation, while neurogenesis was finished in our experiment. Indeed, an increase in pulsatile LHRH release is the critical factor for the onset of puberty; hypothalamic LHRH content shows a steady increase during the first three months of life in male rats, and an increase in pulsatile LHRH release occurs at the onset of puberty in female rhesus monkeys (Terasawa *et al*., 2001).

Our opinion is that LHRH affects the influence of oestrogen on the AMY neurons by altering the distribution and number of receptors in the nuclei of different types of neurons.

List of abbreviations:
AMY – amygdala

ER – estrogen receptors
nucleus medialis – NM
nucleus corticalis – NCO
nucleus centralis – NCE
massa intercalata – MI
nucleus basomedialis – NBM
nucleus basolateralis – NBL
nucleus lateralis posterior – NLP
nucleus lateralis anterior – NLA

ACKNOWLEDGEMENT:
This work was supported by a grant from the Ministry of Science, Technology and Development of the Republic of Serbia, No 1843 (Morphology of organs of experimental animals, little green monkey and their reactivity to hormones)

Address for correspondence:
Dmitar Drekić, DVM, PhD
Bulevar JA 18
11000 Belgrade
Serbia&Montenegro
e-mail: dmitar@vet.bg.ac.yu

REFERENCES

1. *Andrea CG, James LR, Gibson MJ,*1999, Mechanism for the regulation of gonadotropin releasing hormone gene expression in the developing mouse, *Endocrinol,* 140, 5, 2280-7.
2. *Azad N, LaPaglia N, Agrawal L, Steiner J, Uddin S, Williams DW, et al*, 1998**.** The role of gonadectomy and testosterone replacement on thymic luteinizing hormone production, *J Endocrinol,* 158:229-35.
3. *Barnes DC, Sarkar KD,* 1995, The Reproductive Neuroendocrinology, CRC Press, Inc.
4. *Beyer C, Green SJ, Hutchison JB,* 1994, Androgens influence sexual diferentation of embryonic mouse hypothalamic aromatase neurons in vitro, *Endocrinol,* suppl 3, 135, 1220-6.
5. *Dellovade TL, Rissman EF, Thompson N, Harada N, Ottinger MA,* 1995, Co-localization of aromatase enzyme and estrogen receptor immunoreactivity in oreoptic area during reproductive aging, *Brain Res,* 674, 2, 20, 181-7.
6. *Drekić D, Malobabić S, Gledić D, Cvetković D,* 1995a, Different neuronal and glial cell groups in corticomedial amygdala react differently to neonatally administered estrogen, *Neurosci,* 66, 475-81.
7. *Drekić D, Malobabić S, Štimec B, Cvetković D, Lozanče O,* 1995b, Study of neurons and glial cells of basolateral amygdala in male and female rats neonatally treated with estrogen, *Int J Neurosci*, 83, 145-51.
8. *D'Souza SS, Selmin F, Murty SB, Qiu W, Thanoo BC, DeLuca PP,* 2004, Assessment of fertility in male rats after extended chemical castration with a GnRH antagonist, *AAPS PharmSci, 6, article 10.*
9. *Kalra SP, Kalra PS,* 1983, Neural regulation of luteinizing hormone secretion in the rat, *Endocr Rev,* 4, 311-51.
10. *Labrie F, Dupont A, Belanger A, Lacourciere Y, Beland L, Cusan L, Lachance R,* 1990, Complete response to combination therapy with an LHRH agonist and flutamide in metastatic male breast cancer: a case report, *Clin Invest Med,* 13, 275-8.
11. *Malobabić S, Drekić D, Prostran M, 2002,* Autoradiographic topography of estrogen receptors in the amygdala of the male rat, *Acta veterinaria, 52, 107-16*

12. *Mitsushima D, Hei DL, Terasawa E,* 1994, Gamma-aminobutyric acid is an inhibitory neurotransmitter restricting therelease of luteinizing hormone-releasing hormone before the onset of puberty, *Proc Natl Acad Sci USA, 91,395-9* .
13. *Moore JP, Wray S*, 2000, Luteinizing hormone releasing hormone (LHRH) biosynthesis and secretion in embryonic LHRH neurons, *Endocrinol,* 141, 4486-95 .
14. *Narada N, Abe-Dohmae S, Loeffen R, Foidart A, Balthazart J,* 1993. Synergism between androgens and estrogens in the induction of aromatase and its messenger RNA in the brain, *Brain Res,* 622, 243-56.
15. *Stricker HJ,* 2001, Luteinizing hormone-releasing hormone antagonists in prostate cancer, *Urology,* 58, 24-7.
16. *Terasawa E, Keen KL, Mogi K, Claude P*, 1999, Pulsatile release of luteinizing hormone-releasing hormone (LHRH) in cultured LHRH neurons derived from the embryonic olfactory placode of the rhesus monkey, *Endocrinol,* 140,1432-41.
17. *Terasawa E, Schanhofer WK, Keen KL, Luchansky L,* 1999b, Intracellular Ca(2+) oscillations in luteinizing hormone-releasing hormone neurons derived from the embryonic olfactory placode of the rhesus monkey, *J Neurosci,* 19, 5898-909.
18. *Terasawa E, Fernandez DL,* 2001, Neurobiological mechanisms of the onset of puberty in primates, *Endocr Rev,* 22, 111-51.
19. *Tsuruo Y, Ishimura K, Osawa Y,* 1995, Presence of estrogen receptors in aromatase-immunoreactive in the mouse brain, *Neurosci Lett,* 195, 49-52.
20. *Witkin WJ, 1999,* Microscopy research and technique, 44,1, 11-18, CRC Press, INC.

UTICAJ LHRH HORMONA NA RECEPTORE POLNIH HORMONA U JEDRIMA NEURONA NUCLEUSA AMYGDALE

DREKIĆ D, MRVIĆ-JOVIČIĆ VERICA, ZORIĆ Z, DJELIĆ DIJANA, BLAGOJEVIĆ ZDENKA i NIKOLIĆ ZORA

SADRŽAJ

Za dokazivanje uticaja LHRH na distribuciju receptora estrogena u jedrima pojedinih tipova neurona nucleusa amygdale (AMY), korišćeni su mužjaci pacova, tretiranih 83. dana sa 25 μg LHRH i sa 250 μCi H^3- E_2 dva sata pre žrtvovanja. Kontrolna grupa pacova je takođe tretirana sa 250 μCi ^3H-E_2 2 sata pre žrtvovanja. Obe grupe životinja su bile stare 85 dana.

Nakon autoradiografije, u kontrolnoj grupi su proučavana obeležena mesta u AMY. Jezgra AMY sa najvećim brojem receptora estrogena su *nucleus medialis* (NM), *nucleus corticalis* (NC), *nucleus centralis* (NCE) i *massa intercalata* (MI) kortikomedijalnih delova AMY. Ova jezgra pripadaju filogenetski starijem AMY. Manji broj obeleženih jedara neurona je bilo prisutno u *nucleus basolateralis* (NBL), *nucleus basomedialis* (NBM), *nucleus lateralis anterior* (NLA) i *nucleus lateralis posterior* (NLP) koji pripadaju filogenetski mladjem delu AMY mužjaka pacova. Ova distribucija receptora estrogena je biološki pre povezana sa značajnim uticajem

estrogena na regije odgovarajuće divergencije, nego na regije senzorske konvergencije AMY.

Kod tretiranih mužjaka pacova sa 25 μg LHRH i 2 sata pre žrtvovanja sa 250 μCi ^{3}H-E_2 primetili smo drugačiju distribuciju receptora estrogena, sa različitim tipovima nukleusa neurona (nucleus AMY).

Primećeno je takodje da u nukleusima piramidalnih neurona postoji manji broj receptora za estrogene, dok je u fuziformnim i stelatnim neuronima broj receptora bio sličan kao i u kontrolnoj grupi NM, NCO, NCE. U *massa intercalata* našli smo ogroman broj receptora u nukleusima neurona, u starijim delovima AMY. U mladjim delovima AMY, NBL i NLP uočili smo značajan pad broja receptora estradiola u nukleusima piramidalnih i fuziformih neurona. Smatramo da LHRH hormon utiče različito na distribuciju receptora estrogena u jedrima neurona. Oni su najmanje zastupljeni u piramidalnim neuronima, a najviše u stelatnim neuronima nucleusa AMY.