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AUTORADIOGRAPHIC TOPOGRAPHY OF ESTROGEN RECEPTORS IN THE AMYGDALA OF THE MALE RAT

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Autoradiography was used to localize estrogen- accumulating cells in the amygdala (AMY) of seven adult male rats (85 days old). Two hours after injection with ^{3}H-E2, the animals were sacrificed and, after processing for autoradiography, the labelled sites in the AMY were studied. The nuclei of the AMY which showed the highest density of estrogen binding (receptors) were nucleus medialis (NM), nucleus corticalis (NCO), nucleus centralis (NCE) and massa intercalata (MI). 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). Scant 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 in the AMY.

Key Words: amygdala, autoradiography, estrogen receptors, male rats.

INTRODUCTION

Estrogen target cells have been found in the brain of many vertebrates including teleosts, amphibians, reptiles, birds and mammals (Commins and Yahr, 1985; Stumpf and Sar, 1978). Sexual differentiation of male rat brain during the "critical period" which occurs during late fetal and early neonatal development is believed to result from the interaction with estrogen receptors (ER) (Arnold and Gorski, 1984).

The sex steroids, estradiol (E) and testosterone stimulate sexually dimorphic behavioral patterns and regulate gonadotropin secretion in various vertebrates by acting directly on the brain (Commins and and Yahr, 1985; Sibug *et al*, 1991; Simerley, 1996). Several areas of male rat brains have a high density of ER including AMY and the anteroventral periventricular nucleus of the preoptic region (Simerley *et al*, 1996; Shughrue *et al*, 1997; Shughrue *et al*, 1990).

Estrogen enhances neuritic development and plays a role in axonal growth patterns (Sibug *et al,* 1991) in different brain regions such as the neonatal hypothalamus, preoptic area (Naftolin *et al,* 1972), and induces synaptic plasticity in the adult primate brain (Naftolin *et al,* 1993), in the nuclei of AMY (Drekic et al, 1991), prefrontal and parietal cortex (Kolb and Stewart, 1991; Drekic *et al,* 1992) and CA1 pyramidal cells of the hippocampus (Woolley et al, 1993; 1997). Estrogen was shown to influence strongly the development of dendritic arborisation and spines of neurons in rat AMY nuclei (Drekic *et al,* 1991; Simic *et al,* 1991).

Autoradiographic analysis can be a useful tool in the identification of potential sites of estrogen and androgen action (Shughrue *et al,* 1990). In this study we investigated the distribution of radioactive sites in different nuclei of adult male rat amygdala two hours after injection of a single dose of labeled estrogen.

MATERIALS AND METHODS

The presumptive distribution of ER in the AMY complex of adult male rats was determined using an autoradiographic method. Seven male adult rats (85 days old) received an intraperitoneal injection of 60 - 100 μCi tritiated estradiol [^{3}H - E_2] (Amersham, 90-115 Ci/mmole) in 10% ethanol saline. 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 using paraffin embedding. The region of AMY was sliced in 5 μ thick transverse serial sections, which were covered with ILFORD L4 emulsion, and exposed for 5 months at 40 °C. After development with KODAK 19, 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 the investigated amygdaloid nuclei, we classified them, according to the intensity of labeling, as highly, moderately and scantelyl abeled. 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 NU2, Carl Zeiss Microscope (Jena) at a magnification of x 1024.

RESULTS

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

The highest degree of labeling was present the nuclei of the phylogenetically older, corticomedial part of amygdala: in NM, followed by NCE, NCO and MI respectively (Figure 1).

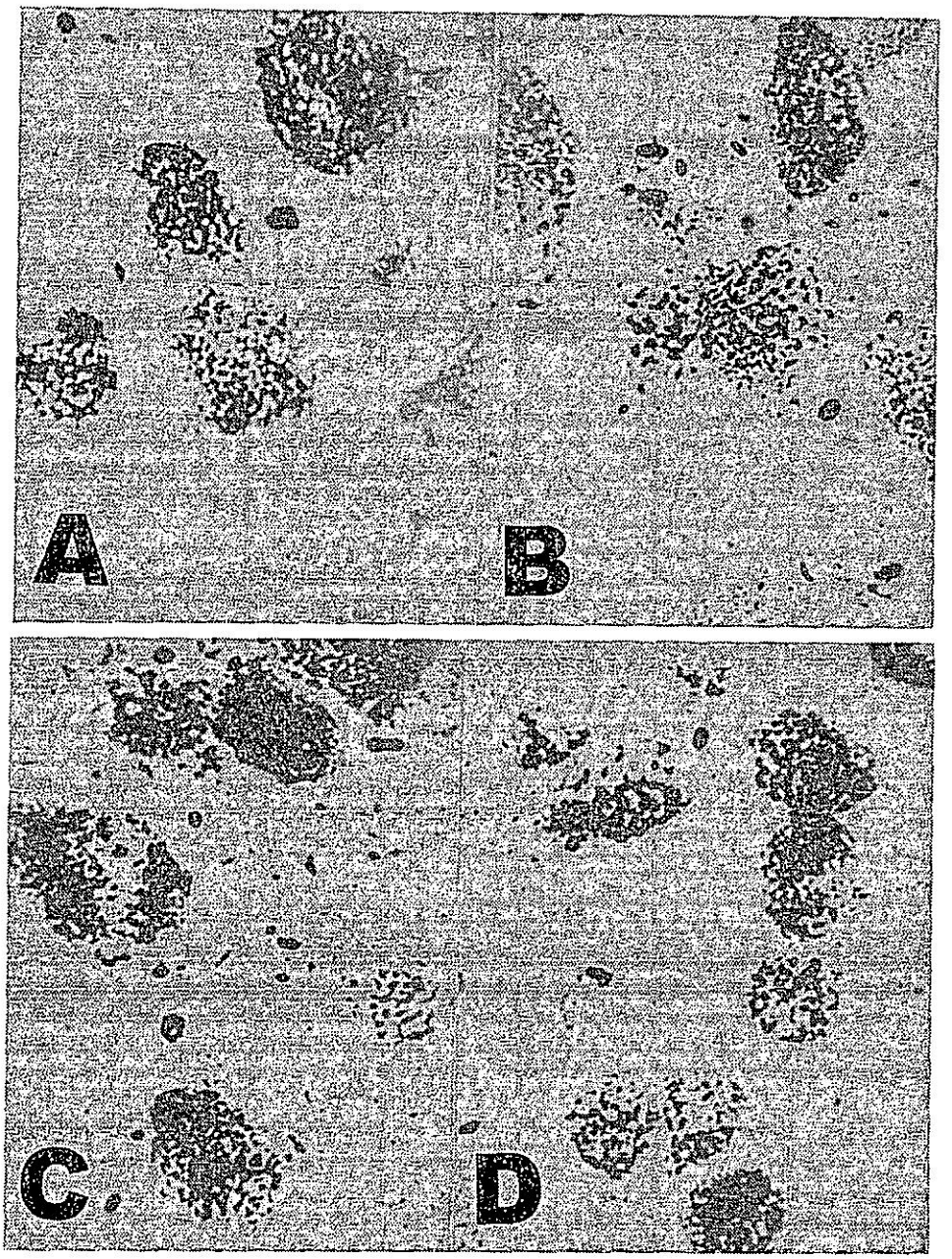

Figure 1. Labeled neurons in AMY of adult male rats (85 days old) treated with tritiated estrogen 2 hours before sacrifice. A - nucleus medialis; B- nucleus corticalis; C- nucleus centralis; D- massa intercalata. Hematoxylin; X 1024. Scale bar = 12 μm

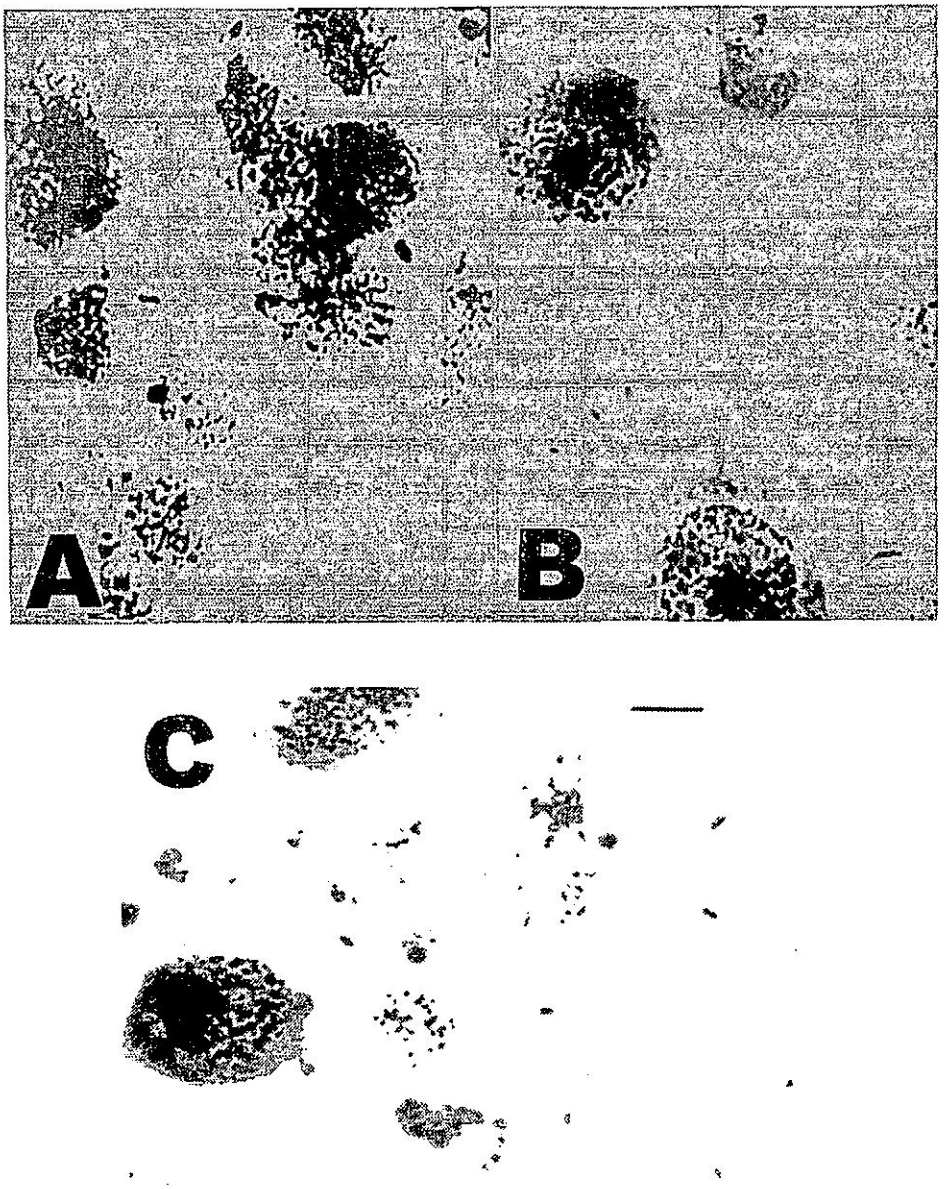

Figure 2. Labeled neurons in AMY of adult male rats (85 days old) treated with tritiated estrogen 2 hours before sacrifice. A - nucleus basomedialis; B- nucleus basolateralis; C nucleus lateralis posterior. Hematoxylin. X 1024, Scale bar = 12 μm

In the phylogenetically younger, basolateral part of AMY, numerous labeled cells (moderate labeling) were also observed in NBM and NBL. (Figure 2).

Few scattered weakly labeled cells were present in the NLA, NLP of the phylogenetically younger, basolateral part of AMY, and in NTLO.

DISCUSSION

The ontogeny and detailed anatomical distribution of ER in the cerebral cortex of the 2 - day - old mouse forebrain and pituitary gland was first described by Shughrue *et al,* (1990). They used 16 - α 15 iodo-11β -methoxy- β17 - estradiol for the following reasons: (a) it binds to the estrogen receptor with an affinity almost indistinguishable from that of estradiol, (b) it has a short autoradiographic exposure time because of its high specific activity (2200 Ci/ mmol) and (c) it has poor affinity for rodent α - fetoprotein (Shughrue *et al,* 1990).

In our study of the ER in the nuclei of the AMY in male rats the highest concentration occured in NM, NC, NCE and NBL. Scattered labeled neuronal nuclei were also present in NLA and NLP of the male rat amygdala. This distribution of hormone receptors, is in agreement with the distribution seen in other mammals, including rats (Commins and Yahr, 1985; Sinchack *et al,* 1996). It is also in agreement with biochemical data showing that male rats bind high levels of E_2 in cell nuclei of the preoptic area, hypothalamus, corticomedial part of AMY, parietal, temporal and entorhinal cortex (Sinchack *et al,* 1996). The distribution of ER in AMY found by the autoradiographic method confirmed the results of our previous investigations. The different reactivity of nuclei of AMY found in adult rats (Drekic *et al,* 1995a; 1995b) corresponds well to the described distribution of ER. In situ hibridization histochemical studies revealed distinct distribution patterns of both ERα and ER β mRNA in the female rat brain (Osterlund *et al,* 1998). Estrogen β receptors were found in central and medial anterodorsal nuclei, while colocalization of both α and β subtypes, occured in the medial posterodorsal nucleus (Osterlund *et al,* 1998). ER α are predominant in rat basal forebrain cholinergic neurons (Shughrue *et al,* 2000).

Two hours after injection of radioactive estrogen we obtained more intensive labeling in the cell nuclei of neurons than in the cytoplasm. This indicates that injected estrogen was efficiently transported into cell nuclei during those two hours. Estrogen has been shown to change the membrane excitability of neurons within minutes (Schumacher, 1990; Smith, 1989). Such rapid effects have been described in numerous regions, including the anterior hypothalamus (Frye et al, 1996; Caldwell *et al,* 1996), medial and other nuclei of the corticomedial and basolateral part of AMY, claustrum, hypothalamic nuclei (Drekic *et al,* 1997), hippocampus, neostriatum (Alonso- Solis *et al,* 1996) and cerebellum (Smith *et al,* 1987;1989). ER α is localized in hippocampal neurons, closely associated with the plasma membrane, i.e. in extranuclear sites. ER α protein, in addition to acting as a transcription factor, may also act as the receptor involved in one or more of the rapid neuronal responses to estrogen (Clarke *et al,* 2000).

However, the localization of radioactivity in nuclei of neurons in the phylogenetically older, corticomedial part of AMY described here is obviously not closely related to these effects of estrogen on the neuronal membrane. Our findings are more related to the genomic activation via intracellular ER. Estrogen

induced protein synthesis is a relatively slow response, taking hours to a day (Pfaff and Mc Ewen, 1983; Pfaff *et al,* 1994). It is mediated via intracellular receptors, which, when bound to estradiol activate a specific DNA target (Ogawa *et al,* 1997; Ruprecht *et al,* 1996). The resulting newly synthesized proteins are transported down the axons (Pfaff and Mc Ewen, 1993) where they are thought to be involved in plastic changes (Pfaff *et al,* 1994). The nuclear receptors offer the opportunity to relate molecular events in neurons to simple instinctive mammalian behavior (Ogawa *et al,* 1996, 1997; Petterson *et al,* 1997; Wagner and Morell, 1996; Ruprecht *et al,* 1996; Baker *et al,* 1996; Shughrue *et al,* 1997). Estrogen- related plasticity has been described in cell populations containing intracellular ER (Stumpf and Sar, 1976; Rees *et al,* 1982; Woolley *et al,* 1993;1997) including the related amygdaloid nuclei (Drekic *et al,* 1991a; Drekic *et al,* 1991b; Drekic *et al,* 1992).

Our findings confirm those obtained by the more sensitive method of in situ hibridization (Osterlund *et al,* 1998). These authors used more detailed terminology in the subdivision of AMY nuclei, wich does not affect comparison with our results. Namely, they found the highest concentration of both α and β ER in NM medialis in female rats, which is the same as we detected by autoradiography in adult male rats. Moreover similarly to us they found the highest concentration of ER α in NM, and the highest concentration of ER β in NC (Osterlund et al, 1998).

We observed that the nuclei of the phylogenetically older, corticomedial part of AMY contain more ER. Considering the main flows of afferent impulses, a very high level of ER was found in intercalated masses and in the central nucleus, i.e. in the "regions of response divergence" of AMY (Maren, 1999). The "regions of sensory convergence" of AMY (Maren, 1999), belonging to the phylogenetically younger basolateral part, contained fewer ER in their neuronal nuclei. We can speculate that the primary regions of sensory convergence (basolateral part) of AMY (Maren, 1999), is under a smaller influence of estradiol, so enabling more "neutral" arrival of information. On the other hand, the essential information processing could be in the intercalated masses and central nucleus of AMY, which contain a greater number of ER. Therefore, these "regions of response divergence" of AMY (Maren, 1999) could be under the greater influence of estrogen levels, suggesting that responses to the same (neutral) information can be different depending on the level of estrogen available to the brain 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 anterior - NLA
nucleus lateralis posterior - NLP
nucleus lateralis anterior - NLA
nucleus lateralis posterior - NLP
nucleus tractus olfactorii lateralis - NTLO

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AUTORADIOGRAFSKA STUDIJA TOPOGRAFIJE ESTROGENIH RECEPTORA U AMIGDALAMA MUŽJAKA PACOVA

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SADRŽAJ

Lokalizacija neurona koji akumuliraju estrogen istraživana je u jedrima amygdala (AMY) sedam odraslih mužjaka pacova (starih 85 dana) metodom autoradiografije. Dva sata posle injiciranja 3H-E2, životinje su žrtvovane i posle autoradiografske obrade proučavana su mesta u amigdalama gde su postojale obeležene ćelije. Jedra u AMY sa najvećom gustinom vezivanja estrogena (receptori) su nucleus medialis (NM), nucleus corticalis (NCO), nucleus centralis (NCE) i massa intercalata (MI). Ova jedra pripadaju filogenetski starijem kortikomedijalnom delu AMY. Srednje ili skromno prisustvo obeleženih neurona bilo je u filogenetski mlađim nucleus basomedialis (NBM) i nucleus basolateralis (NBL). Vrlo malo obeleženih ćelija bilo je u nucleus lateralis anterior (NLA) i nucleus lateralis posterior (NLP) koja pripadaju filogenetski mlađem bazolateralnom delu AMY odraslog mužjaka pacova. Ovakva distribucija estrogenih receptora mogla bi biti u vezi sa biološki signifikantnijim uticajem estrogena na regione divergence odgovora nego na regione senzorne konvergence u AMY.