Neuroendocrinology Letters ISSN 0172–780X

ORIGINAL ARTICLE

Effect of estradiol 17-β on LH subunits and prolactin mRNAs expression in the pituitary of old female rats

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Submitted: *October 11, 2000*
Accepted: *October 23, 2000*

Key words: **LH subunits mRNA expression; LH release; PRL mRNA expression; PRL release; pituitary; old rats.**

*Neuroendocrinology Letters 2000; **21**:431–436 pii: NEL210600A01 Copyright © Neuroendocrinology Letters 2000*

Abstract

OBJECTIVES: The aim of the study was to examine susceptibility of the pituitary gland to estrogenic impulse in old, noncycling rats by measurement of steady state level of mRNAs encoding LH subunits α and β and mRNA for PRL.

METHODS: 22-month-old rats were ovariectomized and after one week they were subcutaneously implanted with silastic tubing filled with oil or with estradiol 17-β. Pituitary α, LHβ and PRL mRNAs content and serum LH and PRL concentration was determined.

RESULTS: The effect of E_2 treatment was manifested by the significant increase in the weight of the uterus and pituitary gland as well as by elevation of total pituitary RNA (109%, 60% and 78%, respectively; $p<0.001$). No significant changes ($p>0.05$) in serum LH concentration were observed, while levels of mRNAs encoding α and LH-β subunits were lowered by 54% ($p<0.05$) and 96% ($p<0.01$), respectively, in the rats subjected to E_2 stimuli. No direct correlation between synthesis and release of LH in E_2 treated old rats was observed. The blood PRL concentration and the pituitary level of PRL mRNA increased up to 2,000% and 1,300%, respectively ($p<0.001$). Spontaneous pituitary adenoma was observed in about 30% of the rats, irrespective of treatment.

CONCLUSIONS: These data show that in old rats estrogenic stimulus can effectively diminish both pituitary LH subunits mRNAs as well as stimulate pituitary PRL mRNA level indicating that the E_2-dependent processes involved in the regulation of corresponding genes are still functional.

Introduction

The gonadal steroid, estradiol 17-β (E_2) plays an important role in the regulation of the biosynthesis and release of gonadotropines as well as prolactin. It has previously been shown that treatment *in vivo* of ovariectomized young mature rats with E_2 resulted in a decreased transcriptional activity of genes encoding the α and LH β subunits [1] and stimulated PRL gene transcription [2]. The regulatory effect of E_2 is mediated into the target cell via estrogen receptor, a ligand-activated transcriptional factor. The estrogen-receptor complex binds to estrogen response elements in target genes and modifies their transcription. The amount of available estrogen receptor seems to decline with age in both pituitary and hypothalamic structures. Our previous study [3] has shown that the amount of the estrogen receptor was diminished by 40–50% in the anterior hypothalamus and the pituitary in 18-month-old rats compared to young female rats (3–6 months old). It is attractive to speculate that the reduced estrogen receptor concentration in the structures of the hypothalamo-pituitary axis in the old female is associated with a change in the estrogen responsiveness in these structures.

The aim of our study was to assess the susceptibility of the pituitary to estrogenic impulse in old, noncycling rats. The effect of estradiol-17β on the steady-state level of mRNAs coding LH subunits and mRNA for PRL in the pituitary gland as well as LH and PRL concentrations in the peripheral blood of 22-month-old ovariectomized rats were examined.

Materials and methods

Animals

Female Wistar rats (350–360 g) were kept under controlled light (LD 10:14) and temperature (22°C) with free access to pelleted food and tap water *ad libitum*. At the age of 22 months all animals were ovariectomized, and one week later they were randomly assigned into two groups. Fifteen rats were subcutaneously implanted with silastic tubing (inner diameter 0.158 cm outer diameter 0.318 cm; Dow-Corning Corporation, Midland MI, USA) filled with sunflower oil. The other fourteen rats received subcutaneous implants containing 375 μg of estradiol 17-β (E_2; Sigma) in oil. Eight days after implantation, all the animals were decapitated and tissues (pituitary and uterus) were excised and weighed. The anterior pituitary was deep frozen in liquid nitrogen, and stored at –80°C until RNA preparation. Trunk blood was collected and plasma samples were stored at –20°C. In 5 out of 15 ovariectomized rats and 4 out of 14 animals treated with E_2, pituitary adenomas were found. Pituitaries that had visible enlarged adenomas were not used for RNA preparation. All experimental procedures were approved by the Ethics Committee at the Kielanowski Institute of Animal Physiology and Nutrition.

Radioimmunoassay

Rat serum LH and PRL were measured by using RIA kits provided by Dr. A.F. Parlow and NIDDK. Values were expressed in terms of rat LH (RP-3) and PRL (RP-3). The intra-assay coefficient of variation was below 5% for both hormones.

RNA preparation and hybridization

The total RNA was prepared from anterior pituitaries according to a scale-adapted CsCl-guanidinium method [4]. Northern blot analysis of LH subunits was performed as previously described [5] with β-actin mRNA, probed with 1150 bp mouse cDNA used as internal reference to correct for the amount of mRNA loading. The rat prolactin cDNA probe was a gift from Prof. J. Martial and Dr. J.N. Laverriere [6]. All probes were labelled with an Amersham Megaprime labeling kit (Amersham, Arlington Heights, IL) The specific band densities were revealed by autoradiography (X-OMAT-AR films, Kodak) at –80°C and quantified by densitometry using a Hoefer GS 300 densitometer.

Statistical analysis

The results are expressed as mean ± SEM. Statistical evaluation was made using a nonparametric Kruskal-Wallis test [7].

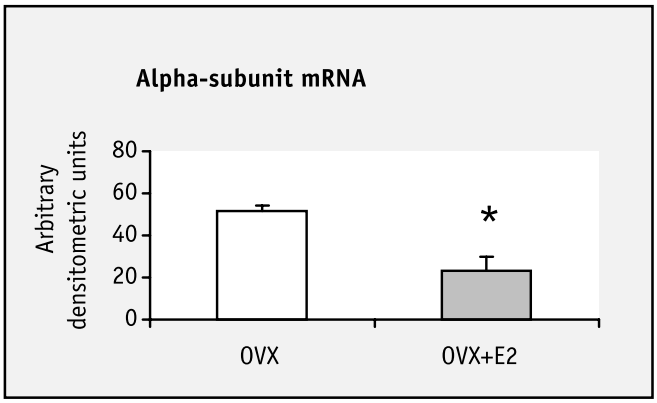
Results

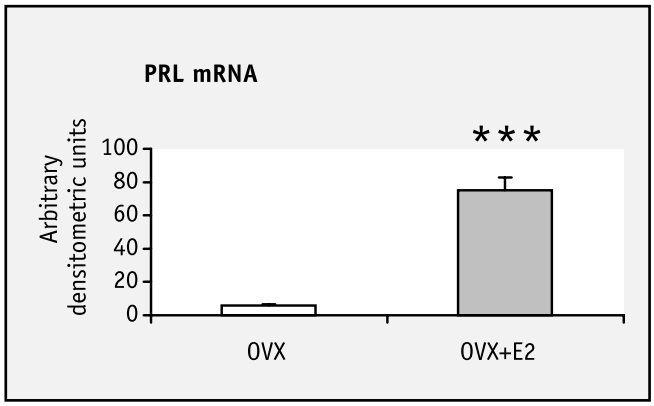
Hormone serum concentrations and LH subunits and PRL mRNAs pituitary levels

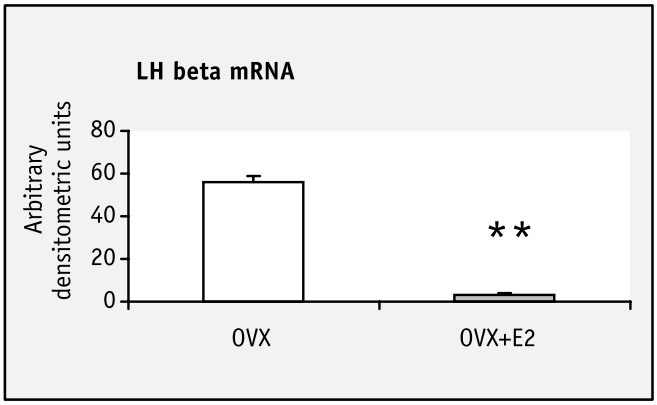
LH concentration was not significantly altered after E_2 treatment; however, the levels of pituitary mRNAs encoding α- and LH-β subunits were significantly depressed by 54% and 96%, respectively (Fig. 1). PRL serum concentration increased by 2,000% in the animals subjected to E_2 treatment whereas pituitary mRNA content for PRL increased by 1,300% (Fig. 2).

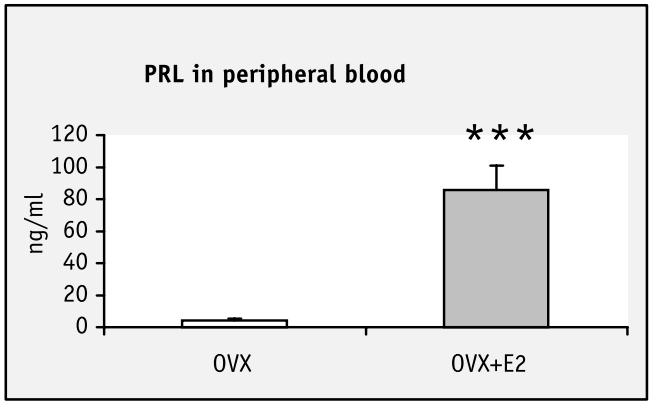
Effect of E_2 on pituitary gland and uterus weight and total pituitary RNA content

Selected tissue parameters in the animals with macroscopically normal pituitary gland are shown in Table 1. Exogenous E_2 increased uterus and pituitary gland weight as well as pituitary RNA concentration by 109%, 60% and 78% respectively, as compared to ovariectomized animals.









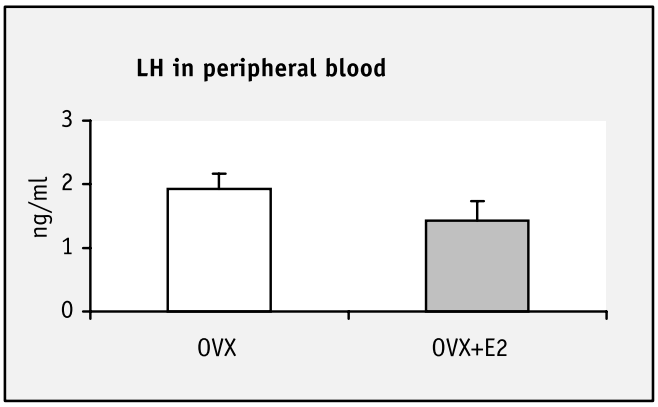


Fig. 1. Levels of α-, LHβ subunit mRNAs in the anterior pituitary and serum LH concentration after E_2 implantation in 22- month-old rats. Ovariectomized rats were implanted with 375 μg of E_2 (OVX+ E_2) or oil only (OVX). Specific mRNAs were assayed by Northern blotting using hybridization with ^{32}P-labelled probes. Absorbance of each band dete cted from the autoradiogram of the RNA blots was determined by scanning densitometry and expressed ad arbitrary densitometry units (ADU). Exposure of films used for densitometric analysis was 24 h for α subunit and 48h for LHβ. Serum LH concentration was assayed by RIA. Values are expressed as mean ± SEM of 10 rats in each group. *p<0.05; **p< 0.01 OVX vs. OVX+E2

Fig. 2. Level of mRNA coding PRL in the anterior pituitary and serum PRL concentration after E_2 implantation in 22-month-old rats. Seven day OVX rats were implanted with silastic tube containing E_2 (375 μg) in oil (OVX+ E_2) or oil only (OVX). Specific mRNAs were assayed by Northern blotting using hybridization with ^{32}P-labelled probe. Filters were analyzed as in Fig 1. The exposure of the films used for the densitometric analysis was 1 h. Serum PRL was assayed by RIA. Values are expressed as mean ± SEM of 10 rats in each group. ***p< 0.001 OVX vs. OVX+E2

Table 1. The effect of estradiol treatment on the selected parameters of reproductive tissues in old rats with normal pituitary gland.

TISSUE PARAMETERS	TYPE OF TREATMENT	
	OVX (n=10)	OVX + E_2 (n=10)
Uterus weight [mg]	510 ± 48	1067 ± 42 ***
Pituitary weight [mg]	12.1 ± 0.4	19.4 ± 0.7***
Total RNA [µg/mg of pituitary]	1.81 ± 0.06	3.22 ± 0.15***

Values are expressed as mean ± SEM; *** p < 0.001 OVX vs. OVX+E_2

Table 2. Tissue weight and the pituitary hormone plasma concentration in old rats with pituitary adenomas.

TISSUE WEIGHT [mg]	TYPE OF TREATMENT	
	OVX (n=5)	OVX + E2 (n=4)
Uterus	534 ± 70	817 ± 50**
Pituitary gland	18.7 ± 3.43	39.3 ± 6.55**
CONCENTRATION OF HORMONES IN BLOOD [ng/ml]		
LH	1.15 ± 0.17	< 0.96
PRL	154 ± 71	351 ± 97*

Values are expressed as mean ± SEM; * p < 0.05; **p< 0.01 OVX vs. OVX+E2

Tissue and hormonal status of old female rats with pituitary adenomas

Frequency of pituitary adenomas appearance was similar (33% in ovariectomized group and 29% in E_2 treated rats). E_2 supplementation increased uterus and pituitary weight by 50% and 110% respectively, as compared to control. In rats with pituitary adenomas mean serum PRL concentration was higher while LH concentration was lower in comparison to their level in rats with normal pituitary glands. The mean concentration of PRL was higher by 128% in rats treated with E_2 (Table 2).

Discussion

In our previous work [1] it was shown that estradiol administered to young ovariectomized rats negatively regulated expression of mRNA gonadotropin subunits *in vivo*. The present study shows that E_2 supplementation to old animals also caused significant reduction of pituitary levels of α-subunit (54%) and LHβ (96%) subunits mRNA. It is well established that in the ovariectomized animal model the major phenomenon reflected by increased gonadotropin gene expression and gonadotropin release is the suppression of the inhibitory effect of steroids on GnRH secretion (synthesis and release), which results in hyperstimulation by the neurohormone of pituitary gonadotrophs and, consequently, hyperexpression of gonadotropin genes [8, 9]. The ability of estradiol and other steroids to modulate gonadotropin gene expression has been investigated predominantly on young animal models. In this model steroids were demonstrated to operate either or both directly at the pituitary and indirectly via negative actions at the hypothalamus to depress GnRH secretion [10, 11]. The greater changes after E_2 treatment observed in LHβ mRNA content as compared to those of α-subunit mRNA suggest that estrogen directly or indirectly is a major regulator of LHβ subunit synthesis. Several down and upstream DNA binding transcription factors regulate LHβ gene expression [12] forming tripartite GnRH response element in the rat LHβ gene promoter. Data based on the expression of the equine LHβ promoter in αT3-1 cells show that GnRH induces transcriptional factors with opposite actions: firstly, Egr-1, acting as a stimulator in conjunction with SF-1 and secondly, Nab1, acting as an inhibitor of Egr-1 [13]. Whether steroids could regulate the expression of gonadotropin genes directly, at the pituitary level, has been intensively investigated [11]. Specific 15-base palindromic LHβ gene region was identified as estradiol responsive element (ERE)

necessary and sufficient to confer *in vitro* E_2 mediated transcriptional response [14]. No such ERE has been identified on α-subunit gene; however, despite the absence of high affinity binding site for the estrogen receptor it has been shown that E_2 inhibits transcription of the human glycoprotein hormone α subunit gene [15]. Moreover, tissue specific expression of the glycoprotein hormone α subunit gene in pituitary gonadotrope has been shown to rely on gonadotrope-specific element (GSE) which binds the orphan nuclear receptor steroidogenic factor-1 [16]. So far there are no detailed studies on the old animal model which explore the possible effect of aging on interactions of differential transcriptional factors with specific genes coding gonadotropin subunits mRNAs. Reduced ability of GnRH to stimulate LH release from pituitary glands of old female and male rats both *in vivo* [17] and *in vitro* [18] have been demonstrated which may reflect decreasing with age sensitivity of GnRH receptors for its stimulation and in consequence markedly affect LH biosynthesis. Strong inhibitory effect of estradiol on LH subunits mRNA pituitary level as well as its powerful hypertrophic effect exerted on pituitary and uterus tissue weight observed in old ovariectomized rats suggest that gene and tissue estradiol sensitivity is not abolished in old animals. Nevertheless, in old female mice age-related changes in estradiol receptor dynamics in hypothalamus, pituitary and uterus have been reported which result in suboptimal reaction of target tissues to estradiol [19, 20]. Inhibitory effect of estradiol on LH subunits mRNA expression was not accompanied by its parallel influence on mean plasma levels of circulating LH. Such result may reflect a decline with aging of the negative feedback loop exerted by E_2 [21] as well as diminished ability of old pituitary to release gonadotropin. Moreover, it also suggests that the effect observed at the transcription level (less mRNA for LH α subunit) may not adequately "translate" to parallel changes observed at the level of mature, biologically active hormone (less concentration of circulating LH). The steady-state level of cytoplasmic mRNA is determined by the balance between synthesis, processing, transport and degradation processes. Also stability of some mRNA species can be changed in response to hormonal induction and this process can play an important role in the control of gene expression and translation efficiency.

Changes in PRL gene expression and synthesis were opposite to those of LH subunits in response to E_2 treatment. It has been shown that E_2 induced increased expression of the PRL gene. The estrogen-receptor complex binds to an estrogen response element in the PRL gene and modifies its transcription. An ERE sequence has been identified in the distal promoter region of the PRL gene [22]. The estrogen responsiveness of the rat PRL gene expression requires not only the presence of the estrogen receptor but also the tissue-specific transcription factor, Pit-1 protein [23]. Increased level of pituitary PRL mRNA is accompanied by high concentration of serum PRL. In young animals PRL may rise 10- to 20-fold after *in vivo* E_2 administration [24]. In our study a similar effect of E_2 on the transcription process of the PRL gene and the secretion PRL in old rats was observed. In many cases the main source of elevated level of PRL in aging rats are adenomas developing in their pituitaries [25, 26]. It is well established that PRL exerts suppressive effect on LH release. Increasing activity of hypothalamic dopaminergic neurons affected frequency and amplitude of LH pulses [27] as well as reduced mean level of circulating gonadotropines and pituitary GnRH receptor numbers [28]. Also stimulation of hypothalamic endorphinergic neurons observed in hyperprolactinemic rats resulted in inhibition of GnRH release [29]. A direct effect of PRL on the pituitary gland cannot be excluded [30] as PRL inhibited LH release from gonadotrope cells *in vitro* and inhibited their sensitivity to exogenous GnRH stimulation.

In summary, our results show that in old rats estrogenic stimulus can effectively diminish both pituitary LH subunit mRNAs as well as stimulate pituitary PRL mRNA level indicating that the E_2-dependent processes involved in the regulation of corresponding genes are still functional.

Acknowledgments

The authors wish to express their warmest thanks to Dr. A.F. Parlow and NIDDK, NHPP Programme for providing us with the RIA kit for LH and PRL (supplied to Dr. K.Kochman, PAN, Jablonna).

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