Changes in Estrogen Receptor ERα and ERβ Expression in Chicken (Gallus domesticus) Adrenal Gland during Short-fasting and Refeeding*

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Estrogen receptors have been found in the adrenal gland of rodents, monkeys, mares and sheep, indicating a connection between sex steroids and the activity of the adrenal gland. In the present study, the expression of estrogen receptors alpha (ERα) and beta (ERβ) in the chicken adrenal gland during stress induced by 24 h fasting and after refeeding was determined using reverse transcription and the polymerase chain reaction (RT-PCR). The presence of both ER mRNAs in the adrenal gland of all examined groups was found. The relative expression of ERα mRNA was higher than ERβ mRNA. There were no significant differences in ERα mRNA expression among the examined groups. On the contrary, we observed changes in ERβ expression during stress conditions. These findings indicate different pathways of estrogen action in the avian adrenal gland. Furthermore, changes in ERβ level suggest that this form of estrogen receptor plays a predominant role for estrogen action in the chicken adrenal gland during stress.

Key words: ERα, ERβ, adrenal gland, RT-PCR, chicken, stress.

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Estrogens play a key role in the regulation of reproductive functions in reptiles (LANCE & BOGART 1992), birds (ICHIKAWA et al. 2003) and mammals (HALL et al. 2001). In birds, estrogens are synthesized by the theca cells of the ovarian follicles (KATO et al. 1995) and they are involved in ovarian formation, differentiation of the oviduct (PALMITER & MULVHILL 1978) and demasculinization of the brain during early life (BALTHAZART et al. 1996). Moreover, estrogens increase adrenal gland activity in rats (SARUHAN & OZDEMIR 2005). On the other hand, the release of corticoids from the adrenal gland affects the reproductive system by activation of the hypothalamic-pituitary-gonadal (HPG) axis (TILBROOK et al. 2000).

Biological effects of estrogens are mediated through two forms of receptors, alpha (ERα) and the more recently discovered beta (ERβ) (KUIPER et al. 1996; PETTERSSON & GUSTAFSSON 2001). These receptors are encoded by two different genes and regulate the expression of E2 target genes by direct binding with a specific DNA domain – estrogen-responsive element (ERE) (KLINGE 2000). In birds, both forms of ER mRNAs have been found in the neuroendocrine system, the liver, ovary and oviduct (ICHIKAWA et al. 2003; HRABIA et al. 2008).

So far, research in molecular biology has been mainly based on mammalian organisms such as the rat, mouse and monkey. Therefore, expression of ERs has been shown in the adrenal gland of rats (KUIPER et al. 1997), monkeys (HIRST et al. 1992), mares (ALM et al. 2009) and sheep (VAN LIER et al. 2003). Even though the chicken (Gallus domesticus), as well as mammals, is an important organism for biomedical research, development and aging, there is no information about ER expression in the avian adrenal gland. The adrenal gland plays a role in the integration of metabolic activity and energy balance, implicating feeding as a major regulator of rhythms in the hypothalamic-pituitary-adrenal (HPA) axis (DALLMAN et al. 1999). Physiological stress, such as starvation, leads to substantially increased release of cortisol.

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from the adrenal cortex (SEED et al. 2000). This pathway often has an inhibitory effect on the reproductive system (TILBROOK et al. 2000). Therefore, the aim of the present study was to determine mRNA ERα and ERβ presence in the chicken adrenal gland and changes in the level of ERs during stress induced by feed withdrawal and after refeeding.

Material and Methods

Animal experiment

The experiment was conducted according to a research protocol approved by the Local Animal Ethics Committee in Cracow (No. 49/OP/2004). Immature (15-week-old) Hy-Line Brown hens (N=18) were kept in individual cages under a light regimen of 14 h light and 10 h dark (lights-on at 0800 h and off at 2200 h) with free access to water and commercial food (DKMII). All chickens were decapitated, and the liver (used as a positive control) and the adrenal gland tissues were isolated, quickly placed into RNAlater and then allowed access to food for 24 h (refeeding).

The birds were divided into three equal groups: (i) with feed and water ad libitum (control), (ii) fasted for 24 h (short fasting) and (iii) fasted for 24 h and then allowed access to food for 24 h (refeeding). All chickens were decapitated, and the liver (used as a positive control) and the adrenal gland tissues were isolated, quickly placed into RNA later and stored in -20°C until total RNA extraction.

The chemicals were purchased from the following companies: TRI-reagent (MRC, Inc., Cincinnati, OH, USA), RevertAid M-MuLV Reverse Transcrip tase, Ribonuclease inhibitor, dNTP mix, MgCl₂, Pol Taq DNA Polymerase, buffers, molecular weight marker – 100 bp DNA ladder (Fer mentas, Vilnius, Lithuania), primers, oligo-dT₁₈ (IBB, Warszawa, Poland). All other reagents were obtained from ICN Biomedicals (Aurora, IL, USA) or Sigma (St. Louis, MO, USA).

RNA isolation and polymerase chain reaction

Total RNA was extracted from the liver and adrenal tissues using the TRI-reagent according to the manufacturer’s recommendations. 2 μg of total RNAs isolated from each tissue were reverse transcribed with RevertAid M-MuLV reverse transcriptase (200U) and oligo-dT₁₈ primers (0.5 μg). As a negative control, untranscribed tissue RNA (reverse transcriptase omitted) was used. RT products (1 μl) were amplified in a Thermocycler Gradient (Eppendorf, Germany) according to Hrabia et al. (2008) in a 12.5 μl reaction mixture containing 1.25 μl of buffer (100 mmol Tris-HCl, pH 8.8, 500 mmol KCl, 0.8% Nonidet P40), 0.312 unit pol Taq DNA polymerase, 0.2 μmol sense and antisense primers, 0.2 mmol each dNTP, 1.5 mmol MgCl₂, and water. PCR conditions were as follows: the initial denaturation for 5 min at 95°C (ERα, GAPDH) or 4 min at 94°C (ERβ), then 30 s at 95°C (ERα, GAPDH) or 30 s at 94°C (ERβ), 30 s (ERα, ERβ) or 15 s (GAPDH) at the annealing temperature, and 30 s at 72°C. Amplifications were completed with an additional extension at 72°C for 7 min. Primers, number of cycles and annealing temperatures for ERα, ERβ and GAPDH are described in Table 1. Negative control (water) was included in all reactions. All PCR products were analysed using agarose gel electrophoresis with a 1.5% agarose gel in TBE buffer (0.5X TBE) containing 1.25 μl of buffer (100 mmol Tris-HCl, pH 8.8, 500 mmol KCl, 0.8% Nonidet P40), 0.312 unit pol Taq DNA polymerase, buffers, molecular weight marker – 100 bp DNA ladder (Fermentas, Vilnius, Lithuania), primers, oligo-dT₁₈ (IBB, Warszawa, Poland). All other reagents were obtained from ICN Biomedicals (Aurora, IL, USA) or Sigma (St. Louis, MO, USA).

Table 1

<table>
<thead>
<tr>
<th>Gene (GeneBank)</th>
<th>Primer sequence</th>
<th>Amplicon size</th>
<th>Annealing temperature</th>
<th>Number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH (K01458)</td>
<td>F: 5’-GGGAGCATGACAGAGGTG-3’&lt;br&gt;R: 5’-AACGCCCTGAGCAATGCT-3’</td>
<td>349 bp (635-983)</td>
<td>52°C</td>
<td>28</td>
</tr>
<tr>
<td>ERα (X03805)</td>
<td>F: 5’-GTCCTTAAATGCACATCATC-3’&lt;br&gt;R: 5’-GGCTCCAGCAGCTCCAGTAA-3’</td>
<td>300 bp (1522-1821)</td>
<td>58°C</td>
<td>30</td>
</tr>
<tr>
<td>ERβ (AB036415)</td>
<td>F: 5’-GATATGCTCCTGCGCATAGC-3’&lt;br&gt;R: 5’-CTTCATGCTCAGCACAGTGC-3’</td>
<td>304 bp (1374-1677)</td>
<td>55°C</td>
<td>30</td>
</tr>
</tbody>
</table>
Results

The presence of ERα and ERβ mRNAs was found in the liver (a positive control) and in the adrenal tissue of all examined groups of chickens. The products were 300, 304 and 349 bp for ERα mRNA, ERβ mRNA and GAPDH mRNA, respectively, and corresponded to the approximate size for each as predicted (Fig. 1).

The relative expression of ERα was significantly higher than ERβ in the control (0.51 ± 0.066 vs. 0.24 ± 0.033) and in the fasted birds (0.44 ± 0.034 vs. 0.18 ± 0.044). In the case of the adrenal gland of the chickens after refeeding, differences between expression of ERα mRNA and ERβ mRNA were insignificant (0.55 ± 0.044 vs. 0.46 ± 0.076). There were no significant differences in ERα mRNA expression among the examined groups (0.51 ± 0.066 vs. 0.44 ± 0.034 vs. 0.55 ± 0.044 in control, short-fasting and refeeding group respectively) (Fig. 1).

With respect to ERβ there was no difference in mRNA expression between the control and fasted birds (0.24 ± 0.033 vs. 0.18 ± 0.044), whereas in the chickens after refeeding the expression was significantly elevated by 162% and 94% compared to the fasted and control chickens, respectively (Fig. 2).

Discussion

Estrogen receptors ERα and ERβ were found in the adrenal gland of several species such as rodents and primates (WEISS & RUO-JUN XU 1990; HIRST et al. 1992). Furthermore, data from many experiments indicate that gonadal hormones have a direct effect on the physiology of the adrenal tissue. For instance, female sheep secreted more cortisol after exogenous ACTH treatment than male sheep. Gonadectomy in these animals reduced the sex differences, suggesting a role for circulating gonadal steroids in the regulation of cortisol secretion at the adrenal gland level (VAN LIER et al. 2003).

SARUHAN and OZDEMIR (2005) received similar results in rats with bilateral ovariectomy, i.e. a decrease in the activity of the adrenal cortex. In contrast, estrogen supplementation causes a significant increase in the activity of the adrenal cortex and medulla. LO et al. (2000) indicate that estrogens may enhance corticosterone feedback by stimulating corticos-
terone production at the adrenal gland or by reduc-
ing corticosterone metabolism.

We reveal the presence of two transcripts (α and β) of ER in the chicken adrenal gland. The presence of both ERs indicates different pathways of estrogen action in the avian adrenal gland. These findings correspond to previous observations implying distinct pathways of estrogen action (LINDNER et al. 1998; PRINS et al. 1998). GUSTAFSSON (1999) assumed that ERα and ERβ differentially expressed in several tissues have varied or even opposite biological actions.

Furthermore, we demonstrated a significant difference between ERα and ERβ levels. A markedly higher expression of ERα mRNA suggests that this type of estrogen receptor is mainly involved in the modulation of adrenal functions in chickens. This is in accordance with a study in the rat that showed higher expression of ERα mRNA than ERβ mRNA in the adrenal gland (KUIPER et al. 1997). On the other hand, we observed changes in ERβ expression level after stress conditions while there were no significant differences in ERα among control, short-fasting and refeeding group. These data indicate that ERβ is predominantly involved in the modulation of adrenal activity by estrogens in response to stress conditions. This conclusion is consistent with previous studies which have shown that estrogens take part in limiting the responses to physiological stress. For example, estrogen administration to postmenopausal women attenuates cortisol responses to mental stress (LINDHEIM et al. 1992). These data are also consistent with those of KOMESAROFF et al. (1998) who showed that in ovariectomized ewes administration of estrogen at physiological levels decreases glucocorticoid responses to stressors.

The HPA system is the main neuroendocrine pathway in the mediation of physiological responses to stressors. Activation of this axis leads to higher endogenous glucocorticoid levels and further exacerbates the pathologies associated with stressors (McEWEN 1998; BAO et al. 2008). Stress suppresses the reproductive system at various levels: through inhibiting the luteinizing hormone-releasing hormone (LHRH) secretion or repressing LH-induced ovulation and sperm release. In addition, glucocorticoids inhibit the testes and ovaries directly, hindering production of the male and female sex hormones (SWAAB 2003). The interaction between the HPA axis and the hypothalamic-pituitary-gonadal (HPG) axis may act in both ways with reproductive hormones also influencing adrenal function (YOUNG 1995). In rodents basal and stress-induced activity of the HPA axis is higher in females than in males (VIAU et al. 2005). This discrepancy suggests that gonadal steroid hormones might be in part responsible for these sex differences. BEUVING et al. (1989) showed corticosterone depletion during chronic stress. Our suggestion is that estrogens, in the case of corticosterone deficiency, may control the adrenal gland activity through ERβ. The specific structure of avian adrenal gland with cortical tissue intermingled with the medullary tissue also seems to be significant.

It is known that estrogens are well-established mood modulators in both males and females (ARPELS 1996). This may be a consequence of two main receptor systems of estradiol ERα and ERβ. Animal studies support an anxiogenic and depressive effect of ERα activation and anxiolytic and antidepressant of ERβ activation (LUND et al. 2005; WAIF & FRYE 2005; HUGHES et al. 2008). This is in agreement with our studies that showed higher expression of ERβ during the time when an organism returns to “normal” conditions. Estradiol’s overall effect on HPA axis activity may be in part due to impairment in glucocorticoid receptor function thereby impairing glucocorticoid negative feedback (TURNER 1990). ISGOR et al. (2003) showed that in rats, ERβ has an important role in HPA axis activation at the hypothalamus level, and is regulated by circulating corticosterone. Adrenalectomy reduced ERβ mRNA expression in the paraventricular nucleus (PVN), whereas corticosterone replacement fully reversed this effect in a dose-dependent fashion. On the other hand, local delivery of estradiol or ERα agonist to the PVN increases stress-induced plasma corticosterone. These findings correspond with our suggestion that ERβ may be an important mediator between the HPA and HPG axis during recovery.

In conclusion, the data presented here clearly show the involvement of estrogens in reaction to stress by changes of ERα and ERβ mRNAs in the chicken adrenal gland. The obtained results indicate that this gland seems to be also a target tissue for estrogen auto-, para-, and endocrine actions.

References


Estrogen Receptors in Chicken Adrenal Gland


