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Estradiol stimulates an anti-translocation expression pattern of glucocorticoid co-regulators in a hippocampal cell model

Sanjana A. Malviya [a], Sean D. Kelly [a], Megan M. Greenlee [a], Douglas C. Eaton [a], Billie Jeanne Duke [a], Chase H. Bourke [b], Gretchen N. Neigh [a,b,*]

[a] Department of Physiology, Emory University, Atlanta, GA, United States
[b] Department of Psychiatry and Behavioral Sciences, Emory University, Atlanta, GA, United States

HIGHLIGHTS

- Estradiol is capable of augmenting corticosterone-induced increases in *Fkbp5* expression.
- Estradiol in combination with a low concentration of corticosterone caused a decrease in expression of *Ppid*.
- Estradiol in combination with progesterone can cause increased expression of *Ppid* in the absence of corticosterone.
- Estradiol in combination with progesterone decreases *Ppid* expression when corticosterone is present.

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ABSTRACT

A consistent clinical finding in patients with major depressive disorder (MDD) is hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis, the system in the body that facilitates the response to stress. It has been suggested that alterations in glucocorticoid receptor (GR)-mediated feedback prolong activation of the HPA axis, leading to the dysfunction observed in MDD. Additionally, the risk for developing MDD is heightened by several risk factors, namely gender, genetics and early life stress. Previous studies have demonstrated that GR translocation is sexually dimorphic and this difference may be facilitated by differential expression of GR co-regulators. The purpose of this study was to determine the extent to which ovarian hormones alter expression of GR and its co-regulators, Fkbp5 and Ppid, in HT-22 hippocampal neurons. The impact of corticosterone (cort), estradiol (E2), and progesterone (P4) treatments on the expression of the genes *Nr3c1*, *Ppid*, and *Fkbp5* was assessed in HT-22 hippocampal neurons. Treatment of cells with increasing doses of cort increased the expression of *Fkbp5*, an effect that was potentiated by E2. Exposure of HT-22 cells to E2 decreased the expression of *Ppid* and simultaneous exposure to E2 and P4 had combinatory effects on *Ppid* expression. The effects of E2 on *Ppid* extend previous work which demonstrated that serum E2 concentrations correlate with hippocampal *Ppid* expression in female rats. The results presented here illustrate that E2 generates an anti-translocation pattern of GR co-regulators in hippocampal cells.

1. Introduction

Chronic stress exposure has been linked to the pathophysiology of multiple diseases and disorders [1]. The hypothalamic–pituitary–adrenal (HPA) axis mediates the stress response and response to stress is terminated by a negative feedback mechanism. In the acute state, the response to stress is adaptive and provides the necessary energy for an organism to cope with the stressor. However, when stress exposure becomes chronic, negative feedback on the HPA axis can become insensitive, leading to prolonged exposure to glucocorticoids, the hormones that mediate the stress response [2].

Negative feedback on the HPA axis is stimulated when circulating glucocorticoids bind to cytosolic glucocorticoid receptors (GR) in the hippocampus, prefrontal cortex, and pituitary gland. Ligand binding to the GR leads to nuclear translocation and subsequent interaction with DNA response elements, ultimately leading to gene transcription and termination of the stress response. Upon binding to glucocorticoids, the GR dimerizes and subsequently translocates into the nucleus, a process that is modulated by several immunophilin co-chaperones. These proteins are postulated to regulate both GR sensitivity to circulating glucocorticoids and its translocation upon ligand binding [3]. FK506 protein 51, also known as Fkbp5, is a normal component of 55% of GR complexes that lowers GR's affinity for glucocorticoids. Ppid, an

* Corresponding author at: 615 Michael Street, Suite 601, Atlanta, GA 30322, United States. Tel.: +1 404 727 9022; fax: +1 404 727 2648.
E-mail address: gretchen.neigh@emory.edu (G.N. Neigh).

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immunophilin peptidyl–prolyl cis trans isomerase also known as Cyp40, competes with Fkbp5 and facilitates GR translocation by increasing GR's affinity for the motor protein dynein [3]. Once in the nucleus, GR activates a multitude of genes, including *Fkbp5* in an ultra-short feedback loop that negatively regulates further GR translocation [4]. Given that Fkbp5 suppresses GR translocation, increased expression of *Fkbp5* is hypothesized to reduce GR negative feedback on the HPA axis. Furthermore, functional polymorphisms of *Fkbp5* lead to higher expression and blunted negative feedback on the HPA axis and have repeatedly been associated with a higher risk for developing mood disorders [5].

The manifestation of stress related disorders such as depression is greater in women than in men [6], and the susceptibility of women is heightened during times of fluctuation in ovarian hormones [7–9]. Rodent studies suggest that these differences first appear during adolescence, with females displaying more depressive behavior after exposure to chronic stress compared to males [10–16]. Furthermore, the differences in stress-induced changes in behavior may be a reflection of sex differences in the stress-induced changes in the HPA axis. Recently, our group documented that both GR translocation and expression of GR co-chaperones differed between male and female rats exposed to chronic adolescent stress. Females with a history of chronic adolescent stress displayed increased depressive-like behavior [10], impaired negative feedback of the HPA axis, and a reduction in GR sensitivity; results that were not observed in males [12].

Sex differences in the effects of stress on GR may be facilitated by gonadal hormones. On a molecular level, gonadal steroids influence the activity of both the GR itself and its co-regulators [11,12]. For example, estradiol (E2) has been shown to reduce GR action, and progesterone (P4) competes with cort for GR binding [17,18]. Additionally, gonadal hormones influence GR co-regulators. In breast cancer cells, E2 has been shown to regulate the expression of *Ppid*, whose gene-product is believed to facilitate GR translocation [19]. Additionally, *Fkbp5* has hormone response elements for androgens and progestins, as well as glucocorticoids [20,21]. Crosstalk in regulation of co-regulators by ovarian steroids suggests a molecular mechanism by which ovarian steroids can modulate GR sensitivity. Taken together, these studies suggest a role of ovarian steroids in the modulation of the GR system. Several studies have examined GR regulation *in vitro* but they have focused on cells of peripheral origin and cell type has been demonstrated to impact the influence of ovarian steroids on co-regulators [22–25]. Further study is therefore necessary to determine if ovarian steroids modulate expression of GR co-regulators in neurons.

Previous work suggests that ovarian steroids influence hippocampal GR regulation more than testosterone [12], therefore, the current study focused on the effects of E2 and P4 on the expression of *Nr3c1*, the gene that encodes GR, and expression of two of GR's co-regulators, *Fkbp5* and *Ppid*. To determine whether E2 and P4 could alter the expression of GR co-regulators in hippocampal cells, we assessed the impact of multiple E2 and P4 concentrations in combination with cort on the expression of *Nr3c1*, *Fkbp5*, and *Ppid in vitro* in HT-22 mouse hippocampal neurons. HT-22 cells represent an ideal model system for these hormone studies, as they express functional GR, PR and both isoforms of the ER [26–28].

2. Materials and methods

2.1. Cell line

HT-22 cells, which are immortalized cell line derived from mouse hippocampi were obtained from Salk Institute (La Jolla, CA). Cells were cultured in media made of Dulbecco's modified eagle medium (DMEM) (Invitrogen, Grand Island, NY) and 10% fetal bovine serum (FBS) (Invitrogen, Grand Island, NY) with PenStrep (Life Technologies) under standard conditions (37 °C, 5% CO_2).

2.2. Hormone preparations

Hormone doses were based on previous *in vivo* observations of serum hormone level in rats during following stress exposure [12]. Prior to each hormone exposure, cells were seeded at a density of 6.2×10^4 into six-well plates using 0.05% Trypsin–EDTA, and allowed to grow for 24 h. The medium was then replaced with a stripped medium containing a phenol red free DMEM/F12 (Invitrogen, Grand Island, NY) and 10% charcoaled-stripped fetal bovine serum (Sigma Aldrich, St. Louis, MO) for 24 h. This medium has minimal hormones, and thus will not confound the effects of exposing cells to hormones. After this period, cells were exposed to various hormone concentrations for a 24 h period. In total, eight wells ($n = 8$) were used for each hormone condition, taken from four separate passages of HT-22 cells. Hormone concentrations used in this study were determined based on peak serum hormone levels observed previously in our lab [12].

The cort solution was prepared by dissolving cort (Sigma Aldrich, St. Louis, MO) in EtOH to make a 29 mM solution (10 mg/mL). Cort was further diluted 1:200 in sterile PBS to make 0.145 mM (50,000 ng/mL) stock solution. The E2 solution was prepared by dissolving β-estradiol (Sigma Aldrich, St. Louis, MO) in EtOH to make 3.7 mM (1 mg/mL) solution. The E2 solution was diluted 1:100,000 in sterile PBS to make a 37 nM stock solution (10 ng/mL). The P4 solution was prepared by dissolving P4 (Sigma Aldrich, St. Louis, MO) in EtOH to make a 3.2 mM (1 mg/mL) solution. The P4 solution was diluted 1:200 in sterile PBS to make 0.016 mM (5000 ng/mL) stock solution. For baseline conditions, EtOH was diluted 1:200 in sterile PBS to make vehicle solution, and control samples were treated with the same amount of this vehicle solution. These stock solutions were added to stripped media to make the appropriate concentrations of hormones.

The hormone concentrations are summarized as follows: In the primary study, cort doses of 0, 50, 400 and 800 ng/mL were applied in conjunction with 0, 20, or 40 pg/mL of E2. Further, cort doses of 0, 50, 400, or 800 ng/mL were applied in conjunction with 0, 10, or 30 ng/mL of P4. In the third study, cort doses of 0, 50, or 400 ng/mL were applied in conjunction with both E2 (0, 20, 40 pg/mL) and P4 (0, 10, 30 ng/mL).

2.3. RT-PCR

After 24 h of hormone exposure, cells were pelleted using 0.05% Trypsin–EDTA. RNA was extracted and RT-PCR was performed as previously described [12]. Briefly, Taqman primer/probe chemistry was used along with Taqman gene expression Master Mix. cDNA was synthesized via AB High Capacity CDNA RT-Kit (Applied Biosystems). Gene expression was quantified for *Nr3c1*, *Ppid*, and *Fkbp5*. *Tfrc* was used as the housekeeping reference gene, and ΔC_t values were calculated in the same manner. Additionally, ΔC_t were normalized to baseline conditions. Changes in gene expression are reported as fold change ($2^{-\Delta\Delta Ct}$).

2.4. Statistical analyses

GraphPad Prism 4.0 and SPSS were used for all statistical analyses in this study, and an alpha value was set to 0.05 for all tests. Sample sizes were as follows: baseline condition (hormone-free), $n = 12$, and for all other conditions, $n = 7$ or 8. In rare cases, wells were removed due to the fact that they did not amplify during PCR which lowered the sample size from 8 to 7. Three-way ANOVAs were conducted to analyze whether P4 and Cort or E2 and Cort had combinatory effects on gene expression. In the cases of the combined effects of E2 and P4, two way ANOVAS were conducted for each of the Cort concentrations followed by Tukey posthoc tests if a main effect was found. If an interaction effect was found, a main effect test was conducted in order to determine the level at which the hormones interacted.