The role of estrogen in hypothalamic regulation of hypothalamus-pituitary-adrenal axis activity, energy homeostasis and bone metabolism

Liu, Ji

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Chapter 3

Intrahypothalamic estradiol modulates hypothalamus-pituitary-adrenal axis activity in female rats
Chapter 3

Intrahypothalamic estradiol modulates hypothalamus-pituitary-adrenal axis activity in female rats

Liu J1,2,3, Bisschop PH2, Eggels L2, Foppen E2,3, Fliers E2, Zhou JN1*, Kalsbeek A2,3

1. CAS Key Laboratory of Brain Function and Diseases, School of Life Sciences, University of Science and Technology of China, P.O. Box 4, Hefei, Anhui, 230026, PR China
2. Department of Endocrinology and Metabolism, Academic Medical Center (AMC), University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands
3. Department of Hypothalamic Integration Mechanisms, Netherlands Institute for Neuroscience (NIN), Meibergdreef 47, 1105 BA, Amsterdam, The Netherlands

Key words: microdialysis, estradiol, HPA axis

Abstract

Estrogen plays an important role in the regulation of the hypothalamus-pituitary-adrenal (HPA) axis, but the neuroendocrine pathways and the role of ER subtypes involved in specific aspects of this interaction remain unknown. In a first set of experiments, we administered estradiol intravenously (i.v.), intracerebroventricularly (i.c.v.) and by intrahypothalamic microdialysis to ovariectomized rats to measure plasma corticosterone (CORT) concentrations from carotid artery blood. Systemic infusion of estradiol did not increase plasma CORT, but i.c.v. estradiol induced a 3-fold CORT increase (p=0.012). Local estradiol infusions in the hypothalamic paraventricular nucleus (PVN) significantly increased plasma CORT (p<0.001). A similar CORT increase was seen after PVN infusion of the estrogen receptor α (ERα) agonist propylpyrazoletriol (PPT), whereas the estrogen receptor β (ERβ) agonist diarylpropionitrile (DPN) had no effect. In a second set of experiments we investigated whether estradiol modulates the HPA-axis response to acute stress by administering estradiol agonists or its antagonist ICI 182,780 (ICI) into the PVN during restraint stress exposure. After 30 minutes of stress exposure, plasma CORT had increased 5.0-fold (p<0.001). Estradiol and PPT administration in the PVN enhanced the stress-induced plasma CORT increase (8-fold versus baseline), whereas ICI and DPN reduced it, as compared to both estradiol and vehicle administration in the PVN. In conclusion, central estradiol modulates HPA-axis activity both in the basal state and during restraint stress. In the basal condition the stimulation is mediated by ERα sensitive neurons, whereas during stress it is mediated by both ERα and ERβ.

Key words: hypothalamus, stress, corticosterone, HPA-axis, estrogen, estrogen receptor
Introduction

Activation of the hypothalamic-pituitary-adrenal (HPA) axis is considered to be a characteristic physiological response to stress. Interestingly, the activity of the HPA axis shows a pronounced sex difference. Compared to male rats, female rats have an increased basal corticosterone (CORT) secretion [1, 2]. In addition, female rats showed a higher stress response than male rats including increased ACTH and CORT responses [2-4]. Moreover, the female menstrual cycle is a modulator of HPA axis activity. In spite of similar ACTH and cortisol plasma levels in the basal condition during the early follicular and mid-luteal phase of the menstrual cycle, there is a pronounced difference in HPA axis responsivity across the menstrual cycle after stress exposure[5, 6]. Estrogen is thought to play a causal role in the gender or menstrual cycle dependent differential HPA axis responses, as ovariectomy (OVX) reduces basal CORT levels, while estrogen replacement restores basal plasma CORT concentrations [7, 8].

Estrogen receptors (ER) are expressed throughout the whole body including the brain. In the hypothalamus, ER expression has been reported in several nuclei, such as the paraventricular nucleus (PVN), the ventromedial hypothalamus (VMH), the lateral hypothalamus (LH) and the arcuate nucleus (ARC) [9, 10]. The PVN represents the central neuroendocrine setpoint of the HPA axis, balancing the secretion of two neuropeptides, i.e., corticotropin-releasing hormone (CRH) and vasopressin (AVP), during stress [11]. In the rat PVN, predominant ERβ expression was found with rather limited ERα expression [9, 12]. In line with these findings, we recently demonstrated an increased PVN expression of ERβ, but not of ERα, mRNA during acute stress [7]. There are many experimental data showing that ovarian steroids increase HPA axis activity [13, 14], although there are also reports indicating that ovarian hormones may decrease certain aspects of the HPA axis stress response[15, 16]. These differential results are perhaps explained by the two distinct receptor systems mediating the actions of estrogen. Initially, ERα was thought to be critical for its reproductive effects [17], while ERβ was thought to be more critical for synaptic plasticity and emotional behavior [18]. Recently, Lund et al reported that administration of an ERα agonist will increase anxiety-related behaviors, with opposite effects of an ERβ agonist [19]. Previously, they already had shown a similar differential effect on the stress-induced plasma CORT surge, by using E2-containing pellets [13].

Our own recent study showed a clear increase of plasma estradiol (E2) in female rats after exposure to acute stress [20]. Interestingly, local estradiol concentrations in the PVN were significantly elevated at the same time [20]. Consistent with this, PVN aromatase mRNA expression increased as well [20]. These data clearly indicated the PVN as a candidate site for estrogenic modulation of HPA axis activity.

In the present study, we investigated further the role of the different ER subtypes in the regulation of HPA axis activity, by using blood sampling and retro-dialysis techniques, under different conditions, i.e. basal and stress conditions.
Chapter 3

Material and methods

Animals

Adult female Wistar rats weighing 230-285g were housed in individual cages with food and water available ad libitum, with a room temperature of 23°C and a 12h:12h light/dark regimen (light on at 7:00 am). All experiments were performed between 11:00am and 13:45pm. Animals were allowed to adapt to the new environment for one week prior to the start of the experiments. Before surgery, rats were anesthetized using a mixture of ketamine and xylazine (100:10 mg/kg IP). Rats were allowed one-week of post-operative recovery before the start of the actual experiment. All experimental procedures were carried out with approval of the Animal Care Committee of the Academic Medical Center (AMC) in Amsterdam (The Netherlands) or the University of Science and Technology in Hefei (PR China).

Surgical procedures

All animals underwent a bilateral ovariectomy (OVX). Silicon catheters were inserted into the right jugular vein and left carotid artery, for intravenous infusions (i.v.) and blood sampling, respectively. With a standard Kopf stereotaxic apparatus, an intracerebroventricular (i.c.v.) guide cannula was placed into the lateral cerebral ventricle. Alternatively, we placed bilateral microdialysis probes next to the paraventricular nucleus of the hypothalamus (PVN) or inside the ventromedial nucleus of the hypothalamus (VMH). The coordinates were: a) i.c.v. - AP:1.0 mm, lateral:1.5 mm and 3.5 mm ventral from dura, b) PVN - AP:1.6 mm, lateral:1.8 mm (angled at 10°) and 9.1 mm ventral from the surface of the bone, and c) VMH - AP:2.5 mm, lateral:2.0 mm (angled at 10°) and 9.0 mm ventral from dura. We used cranioplastic cement to secure the i.c.v. cannula and the microdialysis probes and the jugular and carotid outlets to four stainless-steel screws inserted into the skull. All probe locations were checked in thionin-stained 35 μm cryostat sections after the animals had been sacrificed. Only the animals with a correct probe placement were used for data analysis.

During the experiments, animals were connected to blood-sampling and microdialysis lines, which were attached to a metal collar and kept out of reach from the rats by means of a counterbalanced beam. This allowed all manipulations to be performed outside the cages without handling the animals. The metal collars were attached at least 12h before the actual experiment. Animals were handled and sham blood was sampled (i.e., blood was withdrawn and immediately returned) regularly in the week before the first experiment began to familiarize them with the experimental procedures. As estradiol was introduced into the systemic circulation via the jugular vein catheter, all blood samples were drawn.
Hypothalamic estrogen regulates HPA via a catheter in the carotid catheter. Each withdrawal of a 0.2 ml blood sample was compensated for with the equivalent volume of saline. β-estradiol (Sigma, St. Louis, USA) was dissolved in pure dimethyl sulfoxide (DMSO) and diluted 100 times with saline or Ringer solution. The ERα agonists propyl pyrazole triol (PPT) (displaying a 400-fold selectivity for ERα over ERβ) was bought from TOCRIS (Bristol, UK). The ERβ agonist diarylpropionitrile (DPN) (a highly potent estrogen ERβ receptor agonist with a 70-fold selectivity over ERα) was bought from TOCRIS (Bristol, UK). The agonists and the ER antagonist ICI 182,780 (TOCRIS, Bristol, UK) were dissolved in Ringer solution containing 1% DMSO. The compounds used in our studies were specifically chosen for their unique binding characteristics in previous studies [13, 21, 22]. In these earlier studies, 0.5 μM solutions of the compounds were used for pellet implantation and 10 mM to 20 mM for micro-infusions in the brain. We have not come across earlier studies using reverse microdialysis to administer estrogen agonists. With a measured estradiol recovery efficiency of 0.002% (in vitro), the drug concentrations used in the present study (i.e., 10 μg/ml) resulted in tissue concentrations of around 1.0 nM, which is close to the tissue concentration of estradiol as measured in our previous study [20]. The EC50 value of estradiol is around 0.15 nM for both ERα and ERβ. The EC50 of PPT is 0.2 nM and 80 nM for ERα and ERβ, respectively. The EC50 of DPN is 0.85 nM and 66 nM for ERβ and ERα, respectively. Therefore, the dose we chose for PPT will result in tissue concentrations that will only activate ERα, whereas those of DPN will only activate ERβ. The estradiol dose chosen will activate both ERα and ERβ. The higher dose of estradiol was chosen to investigate the dose dependency of the effect on plasma CORT.

Microdialysis
Dialysis probes were constructed according to procedures described previously [23]. Thin platinum wire (0.05 mm) was inserted into a piece of microdialysis tubing (molecular weight cutoff, 6000 kDa) and bent; each end was then inserted into one of two pieces of 25 gauge hypodermic tubing that had been soldered together. The dialysis tubing was then secured with epoxy. The U-shaped tip of the microdialysis probe was 1.5 mm long, 0.7 mm wide and 0.2 mm thick. The input port of the dialysis probe was connected to a remote syringe via a fluid swivel (375/22; Instech Laboratories, Plymouth Meeting, PA) interconnected with polyethylene tubing. The syringe contained Ringer’s. Perfusion of the microdialysis probes was performed by a syringe pump (Harvard Apparatus, Holliston, USA). Ringer’s perfusion (3 μl/min) was started 1 hr before the start of the drug infusion.

Experiment#1- i.v. estradiol infusion
From t=0 onwards estradiol (3.5 ng/min dissolved in saline containing 1% DMSO) or vehicle (saline containing 1% DMSO) were continuously infused via the jugular vein catheter in OVX animals. Blood samples (0.2 ml) were collected just before the start of the estradiol
infusion at t=0, and t=15 min, 30 min, 45 min, 75 min, 105 min, 135 min and 165 min later via the carotid catheter. Blood samples from the vehicle animals were taken at the same time points.

Experiment#2 – i.c.v. estradiol infusion
A baseline blood sample (0.2 ml) was taken just before the start of the estradiol or vehicle (Ringer with 1% DMSO) infusion at t=0. Estradiol (200 pg/μl) was dissolved in Ringer containing 1% DMSO. The i.c.v. infusion started with a bolus (5.0 μl/5 min) and then continued with 5.0 μl/h (~16.7 pg/min, i.e., 0.5% of the i.v. dose) for 165 min. Blood samples were collected at t=15 min, 30 min, 45 min, 75 min, 105 min, 135 min and 165 min after the start of the infusion.

Experiment#3 – Administration of estradiol, ERα or ERβ agonist in the PVN via microdialysis
Ringer’s dialysis (3.0 μl/min) in the PVN via the microdialysis probes was started at t=-60 min. The perfusion solution was then changed to lower dose estradiol (10 μg/ml, 3.0 μl/min), higher dose estradiol (50 μg/ml, 3.0 μl/min), PPT (10 μg/ml, 3.0 μl/min), DPN (10 μg/ml, 3.0 μl/min) or Vehicle (1% DMSO in Ringer, 3.0 μl/min). A five min bolus infusion (15 μl/min) allowed the drugs to rapidly reach the tip of the microdialysis probes. Blood samples (0.2 ml) were collected just before the start of the estradiol, agonist or vehicle infusion (t=0) and 15 min, 30 min, 45 min, 75 min, 105 min, 135 min and 165 min after the start of the infusion via the microdialysis probes (i.e., after the change from Ringer’s to the estradiol (agonist)-containing solution or vehicle).

Experiment#4 - Administration of estradiol, ERα or ERβ agonist in the VMH via microdialysis
These experiments were identical to the protocol of Experiment#3, with the exception that the microdialsyis probes were aimed at the VMH instead of the PVN.

Experiment#5 - Administration of estradiol, PPT, DPN or ICI 182,780 in the PVN via microdialysis during restraint stress
Ringer’s dialysis (vehicle, 3.0 μl/min) into the PVN via the microdialysis probes was started at t=-60 min. The solution was then changed to estradiol (50 μg/ml), PPT (10 μg/ml), DPN (10 μg/ml), ICI (10 μg/ml) or vehicle (1% DMSO in Ringer) at t=0 min. A five min bolus infusion (15 μl/min) allowed the drugs to reach the tip of the microdialysis probes rapidly. After the bolus infusion, the infusion rate was changed back to 3.0 μl/min and all animals were restrained in opaque plastic cylinders (18.0 cm length and 5.5 cm internal diameter) for 30 min [24], starting from t=0. Blood samples (0.2 ml) were collected at t=-5 min...
before the onset of the restraint stress and at t=30 min after the onset of the restraint stress.

Hormone measurement
Blood samples were immediately chilled on ice in tubes containing a 5 μl solution of heparin, and then centrifuged (at 4000 rpm, 15 min) at 4°C. Plasma was then stored at -80°C until additional analysis. Plasma estradiol concentrations were determined using an estradiol enzyme-linked immunosorbent kit (sensitivity range: 2–1000 pg/ml, inter-assay variation: 6.0%, intra-assay variation: 3.9%) (BioSource, Nivelles, Belgium). Plasma corticosterone concentrations were determined by a radioimmunoassay kit (sensitivity range: 10–9000 ng/ml, inter-assay variation: 6.5%, intra-assay variation: 3.4%) (MP Biomedicals, Eschwege, Germany).

Statistics
For statistical analyses we used SPSS V17.0 software. All results were initially analyzed by analysis of variance (ANOVA) with repeated measures or with a two-way ANOVA. When appropriate, further post-hoc tests were done with one-way ANOVA, and paired or unpaired student t-tests. Statistical significance was defined as p<0.05. Data are expressed as mean±S.E.M.

Results
Effects of i.v. and i.c.v. estrogen administration on plasma CORT
During the i.v. infusion of estradiol there was no significant difference in plasma CORT concentrations compared to the vehicle infused group (Time, F(7,63)=3.9, P=0.001; Group, F(1,9)=0.142, P=0.715; Time×Group, F(7,63)=0.642, P=0.719) (Figure 3.1A). We measured plasma estradiol in the t=0 and t=165min samples of the experimental animals. In the vehicle-infused group, plasma estradiol concentrations were similar before the start of the infusion and at t=165 min (P=0.38), while in the estradiol-infused group there was a significant increase (P<0.001) (Table 3.1). The plasma estradiol level in the estradiol infused group at t=165 min was comparable to that of intact animals (~100 pg/ml, data not show).

Unlike i.v. estradiol administration, i.c.v. infusion of estradiol in OVX animals markedly increased plasma CORT (Time F(7,70)=4.687, P<0.001; Group F(1,10)=9.244, P=0.012; Time×Group F(7,70)=6.485, P<0.001) (Figure 3.1B). I.c.v. administration of estradiol did not affect plasma estradiol concentrations.
Chapter 3

Effect of estrogen administration in the PVN and VMH on plasma CORT

The overall analysis of the microdialysis experiments in the PVN showed a clear effect of estradiol and estrogen receptor subtype-specific agonists on plasma CORT concentrations.

Figure 3.1. Plasma CORT increased during i.c.v. administration of estradiol (n=6 in both groups)(B), but not during i.v. administration (Vehicle, n=5; Estradiol, n=6)(A).

Table 3.1. Plasma estradiol concentrations during systemic and I.C.V. administration of estradiol

<table>
<thead>
<tr>
<th></th>
<th>Systemic infusion</th>
<th>I.C.V.</th>
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<tr>
<td></td>
<td>Veh</td>
<td>Estradiol</td>
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<tr>
<td>Before infusion (pg/ml)</td>
<td>43,06±4,47</td>
<td>41,86±6,66</td>
</tr>
<tr>
<td>After infusion (pg/ml)</td>
<td>49,53±16,20</td>
<td>104,24±32,81a</td>
</tr>
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a P<0,001, vs before infusion in the same group
The effects of Time $F(7,210)=7.65$, ($P<0.001$), and Group $F(4,30)=16.512$, ($P<0.001$) as well as Time$\times$Group $F(28,210)=3.074$, ($P<0.001$) were very significant (Figure 3.2A). Post-hoc analysis showed that, compared to the vehicle group, all groups, except the DPN-infused animals, exhibited a significant Time$\times$Group interaction ($P<0.001$). Only the high dose estradiol group (50 μg/ml) and the PPT group also showed a significant Group effect ($P \leq 0.001$). Plasma CORT increased already 15 min after the start of the infusion during both the high dose of estradiol (50 μg/ml) and PPT ($P<0.001$). However, the increase in plasma CORT during PPT was transient and had returned to baseline at 165 min, while plasma CORT increased further in the 50 μg/ml estradiol group. In the 10 μg/ml estradiol group, the plasma CORT increase was slower and did not reach significance until 135 min after the start of the estradiol infusion ($P<0.001$). In contrast to the PVN, administration

Figure 3.2 Plasma corticosterone increased during administration of estradiol or PPT in the PVN (A), but not during administration in the VMH (B). In PVN group, Vehicle n=7; 10 ug E2 n=6; 50 ug E2 n=6; PPT n=9; DPN n=8. In VMH group, Vehicle n=7; 10 ug E2 n=6; 50 ug E2 n=8; PPT n=6; DPN n=5.
of estradiol, PPT or DPN in the VMH had no effect on plasma CORT (no significant effects of Time $F(7,189)=1.514$, (P=0.165), Group $F(4,27)=1.159$, (P=0.351) or Time×Group $F(28,189)=0.915$, (P=0.593)) (Figure 3.2B).

**Effect of estradiol and estrogen (ant)agonist administration in the PVN on plasma CORT during restraint stress**

As it was not possible technically to take blood samples via the jugular catheter when the animals were under restraint conditions, only pre-stress and post-stress samples were taken. Restraint stress significantly increased plasma CORT in vehicle, estradiol, E2-agonist and ICI-treated animals. ANOVA analysis showed an overall significant effect of Stress $F(1,26)=254.11$, (P<0.001), but also the effects of Group $F(4,26)=11.13$, (P<0.001) and Group×Stress $F(4,26)=11.33$, (P<0.001) reached statistical significance (Figure 3.3), indicating that the increase in CORT significantly differed between treatment groups. Post-hoc analysis showed that restraint stress elevated plasma CORT concentrations significantly in all 5 groups (p<0.001). After 30 min of stress, estradiol and ERα agonist-infused animals had significantly higher plasma CORT concentrations than vehicle-infused animals (P=0.008 and P=0.001). Interestingly, the ERβ-agonist and ICI-infused animals showed significantly lower plasma CORT concentrations after 30 min of restraint stress compared to the vehicle-infused animals (P<0.001 and P=0.002).

**Figure 3.3** Administration of estradiol or PPT in the PVN increased plasma CORT in response to stress. Administration of DPN or ICI decreased plasma CORT in response to stress. a) Significantly higher than pre-stress levels (P<0.001); b) Significantly higher than vehicle stress response (P<0.05); c) Significantly lower than vehicle stress response (P<0.05).
Discussion

In the present study we observed a clear effect of E2 on HPA axis activity in OVX female rats after i.c.v and PVN administration, but not after a systemic infusion or local administration in the VMH. Under basal conditions, the estrogenic effect was mainly mediated via ER\(_\alpha\)-sensitive neurons in the area of the PVN. During stress conditions, both ER\(_\alpha\) and ER\(_\beta\) in the area of the PVN were involved in the regulation of HPA axis activity, with ER\(_\alpha\) mediating a stimulatory effect and ER\(_\beta\) mediating an inhibitory effect.

The modulatory role of estrogen on HPA-axis activity has been clearly established [1, 25]. The first clear evidence for dichotomous effects of estrogen via opposing roles of the ER\(_\alpha\) and ER\(_\beta\) was provided by the ER-specific knockout mice [17, 18]. Initially it was thought that the distinct ER subtypes were involved in different functions, with ER\(_\alpha\) being mainly involved in reproductive behaviors and ER\(_\beta\) in non-reproductive behaviors [26, 27]. More recently, however, Lund et al. nicely demonstrated that opposing roles of the ER’s are also evident within one behavioral component. They showed that peripheral administration of an ER\(_\beta\) agonist decreases anxiety-related behavior, whereas administration of an ER\(_\alpha\) agonist produced opposite effects [19]. Similar effects were found for the anxiety-induced activation of the HPA-axis. Administration of the ER\(_\beta\) agonist decreased the stress-induced plasma CORT response and administration of the ER\(_\alpha\) agonist increased it. Later, the same group reported that the different ER effects on plasma CORT were mediated by the hypothalamus, since hypothalamic pellets containing the ER\(_\alpha\) agonist increased stress-induced c-fos expression in the PVN, whereas a similar administration of the ER\(_\beta\) agonist decreased it [21]. Our own previous study demonstrated an increased local production of estradiol in the hypothalamus during acute stress [20]. Together these pieces of evidence suggested an essential role for the PVN in estrogen effects on HPA axis activity. The use of reverse microdialysis and permanent vascular catheters allowed us to investigate rapid changes in plasma CORT during estradiol administration into the systemic circulation, the brain ventricular system, or locally within the hypothalamus, without additional stress for the animals. The results of the current study extend the previous results in that now it is clear that changes in hypothalamic E2 availability can acutely change HPA-axis activity, both during basal and stress-activated conditions. Moreover, the effect is specific for the PVN and not for the VMH.

Our first experiment did not yield any significant differences in plasma corticosterone levels after i.v. administration of estradiol or vehicle. However, the central administration of estradiol via an i.c.v. cannula did produce a clear difference. These data indicated that the estrogenic pathway probably is an important component of the central brain mechanisms controlling basal HPA-axis activity. Conversely, these results seemed counterintuitive as it is likely that the i.v.-infused estradiol will also reach these central targets. The most straightforward explanation seems to be that the i.v. concentration we used was too low to activate the HPA axis. However, the physiological concentrations reached with the
current infusion parameters argue against this explanation. An alternative explanation is that the i.v. infusion reaches additional targets, which inhibit activation of the HPA-axis, overruling its stimulatory effect via the ventricular compartment. Since the i.c.v. infusion was able to activate the HPA-axis, most likely via the PVN as shown by the microdialysis experiments, these inhibitory targets of the i.v. infusion should be either peripheral or quite distant from the ventricular compartment. For instance, the prefrontal cortex, which, unlike the hypothalamus, is quite a distance away from the ventricular compartment, and has a profound inhibitory effect on HPA axis activity [28]. At first sight, our results may also seem at variance with previous studies reporting that systemic administration of estradiol does regulate basal HPA-axis activity [29-31]. However, the experimental design of these studies included subcutaneous injections of estradiol in OVX animals for 4-6 weeks, while in the present study we administered estrogen i.v. for just 3 hours. We observed a rise in plasma CORT concentrations after E2 treatment at t=15 min. Importantly, a similar increase at the same time point was present, in the vehicle group. Therefore, we suspect this short surge of CORT release to be induced by DMSO or by the infusion itself, rather than by E2. Indeed, it has been shown previously that DMSO may affects plasma CORT concentrations [32]. We did not observe a similar phenomenon during the i.c.v. experiment, most likely because the total amount of DMSO infused during the i.c.v. experiment was much lower (0.05 μl/hr) than during the i.v. infusion (5.0 μl/hr).

After having established the central effect of estrogen, we focused on the hypothalamus. Effects of estrogen have been reported in various hypothalamic nuclei, including the PVN, VMH and arcuate nucleus [33, 34]. Within the hypothalamus, the PVN is the most important nucleus when it comes to determining HPA axis activity. While the PVN contains large numbers of CRH neurons [35], there are no reports showing CRH expression in the VMH. The present design involved infusion of E2 and agonists into the VMH as a negative control. As expected, VMH treatment did not affect HPA-axis activity at all as compared to vehicle infusion, while administration in the PVN mimicked the i.c.v. effects. Our findings highlight that, although both the PVN and VMH show abundant neuronal ER expression [12, 36, 37], the functional differences between the PVN and the VMH in terms of neurotransmitter content and/or connectivity are responsible for the differential effects of estradiol administration on HPA-axis activity in these nuclei.

When we administered the ERα agonist PPT into the PVN, it mimicked the E2 effects both in basal and stress conditions. ERα expression in the PVN seems limited [9, 12], but many ERα positive neurons are found in the immediate surroundings of the PVN [21]. The present effect we observed may be mediated by these neurons in the peri-PVN based on the following evidence: firstly, previous studies have shown that many GABAergic neurons surround the PVN [38, 39]. Among other things, this peri-PVN region contains GAD-IR neurons that project to the parvocellular PVN [40] and are inhibitory to the hypophysiotropic CRH neurons [41]. Secondly, the GABAergic neurons surrounding the
Hypothalamic estrogen regulates HPA

PVN express ERα [21]. Thirdly, E2 attenuates GABA-B responses in hypothalamic neurons [42] and suppresses the GABA_A-mediated inhibition [43]. In the PVN, ERβ-expressing neurons predominate compared to ERα positive neurons. Of the CRH neurons, 13% co-localize ERβ [9, 10, 44]. However, when we infused an ERβ agonist into the PVN under basal conditions, no effect was observed. Interestingly, the ERβ agonist did decrease stress-induced CORT release, suggesting that ERβ-sensitive neurons have an inhibitory function on the HPA axis only under stressed conditions. We previously showed that acute stress elevated ERβ mRNA expression in the PVN [20]. Consistent with this, Suzuki found that treatment with dexamethasone increased ERβ expression in the PVN [45]. This evidence partly explains why the ERβ agonist only affected the HPA axis under stressed conditions. Under basal conditions, the level of ERβ expression appears insufficient to regulate the HPA axis.

Our own previous work demonstrated an increased local production of estradiol in the PVN during acute stress in female rats, as well as an increased ERβ and aromatase mRNA expression [20]. Together with our present data these results indicate that local changes in estradiol bioavailability may play an important role in the fine-tuning of HPA-axis responsiveness during stress exposure. During stress, the E2- and PPT-treated animals showed higher plasma CORT responses, whereas administration of the ERβ agonist caused a decrease of the stress response. However, since E2 will activate both the ERα and the ERβ, a lower response in the E2- versus PPT-treated animals was to be expected. Therefore, we suppose that in the beginning of the stress response (low plasma CORT), only the ERα effect is available, but together with the increased aromatase mRNA expression and increased local availability of estradiol this causes a positive feedback. Once the HPA-axis is activated and ERβ mRNA expression is increased the inhibitory effect of ERβ activation will become apparent. Therefore, the final plasma CORT response is determined by the balance between ERα and ERβ activation. To test this time-dependent effect of ERα and ERβ activation, either more frequent sampling protocols or combined administrations of ERα and ERβ (ant)agonists, should be performed during stress exposure.

In conclusion, we have demonstrated an estrogenic stimulation of the HPA axis at the level of the PVN, mediated via the ERα. The stimulatory effect of local ERα activation is present both in the basal state and during restraint stress, whereas the inhibitory effect of ERβ receptor activation may only become apparent when the HPA-axis is activated.

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Chapter 3

References


24. Marin MT, Cruz FC, Planeta CS. 2007 Chronic restraint or variable stresses differently affect the behavior, corticosterone secretion and body weight in rats. Physiol Behav. 90(1):29-35.


