Published in final edited form as:

Mol Cell Endocrinol. 2012 October 15; 362(1-2): 85–90. doi:10.1016/j.mce.2012.05.012.

Glucocorticoid-induction of hypothalamic aromatase via its brain-specific promoter

DC Brooks, H Zhao, B Yilmaz, VJS Coon, and SE Bulun

Abstract

In the brain, a 36-kb distal promoter (I.f) regulates the Cyp19a1 gene that encodes aromatase, the key enzyme for estrogen biosynthesis. Local estrogen production in the brain regulates critical functions such as gonadotropin secretion and sexual behavior. The mechanisms that control brain aromatase production are not well understood. Here we show that the glucocorticoid dexamethasone robustly increases aromatase mRNA and protein by up to 98-fold in mouse hypothalamic cell lines in a dose- and time-dependent fashion. Using deletion mutants of the brain-specific promoter I.f and chromatin immunoprecipitation-PCR, we isolated a distinct region (–500/+200bp) which becomes enriched in bound glucocorticoid receptor upon dexamethasone stimulation. A glucocorticoid antagonist or siRNA based knockdown of glucocorticoid receptor ablated dexamethasone stimulation of aromatase expression. Our findings demonstrate how glucocorticoids alter aromatase expression in the hypothalamus and might indicate a mechanism whereby glucocorticoid action modifies gonadotropin pulses and the menstrual cycle.

Keywords

aromatase; Cyp19a1; glucocorticoids; hypothalamus; estradiol

1.1 Introduction

The hypothalamic-pituitary-ovarian (HPO) axis governs reproduction in mammals through a variety of mechanisms. Classically, gonadotropin releasing hormone (GnRH) pulses secreted by the hypothalamus coordinate the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. LH and FSH influence the release of the steroids estradiol and the estrogen precursor androstenedione from the ovary in women. Elevated glucocorticoids can result in anovulation; for example, in women with Cushing's disease or in cases of hypothalamic anovulation (e.g. marathon runners), elevated cortisol levels have been associated with amenorrhea (Magiakou, Mastorakos et al. 1997; Kalantaridou, Makrigiannakis et al. 2004). The mechanism underlying anovulation in the setting of high glucocorticoid levels is not well understood.

In the case of hypothalamic anovulation it was proposed that locally elevated estrogen levels in the hypothalamus might lead to the characteristically low circulating levels of estrogen,

© 2012 Elsevier Ireland Ltd. All rights reserved.

Corresponding author: Serdar E. Bulun, MD, George H. Gardner Professor of Clinical Gynecology, Chair, Department of Obstetrics and Gynecology, Feinberg School of Medicine at Northwestern University, 250 E. Superior Street, Chicago, IL 60611, 312-472-3636 (tel), 312-503-3740 (fax) s-bulun@northwestern.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

FSH and LH. In a portion of hypothalamic anovulation cases, stress-induced corticotropin releasing hormone (CRH) and cortisol have been implicated as causal factors in anovulation (Rivier and Vale 1984; Xiao, Luckhaus et al. 1989). Thus, we hypothesized that cortisol or its agonists may regulate hypothalamic aromatase expression, thereby increasing local levels of estradiol and leading to FSH and LH suppression and anovulation. To test this hypothesis *in vitro*, we studied the effects of the glucocorticoid agonist dexamethasone and its transcriptional mediator, the glucocorticoid receptor (GR), in murine hypothalamic cell lines that express aromatase.

Aromatase expression in the mouse is regulated primarily by a proximally located gonadal promoter (PII) and a distally located brain promoter (I.f) (Zhao, Innes et al. 2009). The hypothalamus, hippocampus and amygdala are the primary sites in the brain that express aromatase (Honda, Harada et al. 1996; Honda, Harada et al. 1999). Previously, we demonstrated that estrogen and progesterone regulate the aromatase I.f promoter to modest degrees (Yilmaz, Wolfe et al. 2009; Yilmaz, Wolfe et al. 2011). As hypothalamic aromatase and its product estrogen are the most likely regulators of GnRH released from the hypothalamus, we studied the effects of glucocorticoids and GR on hypothalamic aromatase expression via its brain promoter region I.f, using multiple murine hypothalamic cell lines that express aromatase mRNA via promoter I.f.

2. Materials and Methods

2.1 Cell Culture

Mouse embryonic hypothalamic cell lines were purchased from Cellutions Biosystems Inc. (Toronto, ON Canada). The cell lines (N1, N38, N42) were cultured in Dulbecco modified Eagle medium (DMEM; Gibco, New York, NY) supplemented with 8% fetal bovine serum (FBS; Gibco). Following an 18-hr serum starvation, cells were treated with varying doses of dexamethasone (Sigma-Aldrich, St. Louis, MO), RU-486 (Sigma-Aldrich) or spironolactone (Sigma-Aldrich) dissolved in ethanol or vehicle (ethanol alone) for various times.

2.2 RNA Isolation and qPCR

After treatment, hypothalamic cells were collected and total RNA was extracted using TRI reagent (Sigma-Aldrich) per the manufacturer's instructions. RNA integrity was assessed following electrophoresis on a 1% formaldehyde gel. Reverse transcription (RT) was performed on 1 µg of total RNA using Q-Script (Quanta Biosciences, Gaithersburg MD) per the manufacturer's instructions. qPCR was performed on 1 µL of cDNA on an Applied Biosystems 7900 Sequence Detection System (Applied Biosystems, Foster City, CA) using Power SYBR Green (Applied Biosystems) according to the manufacturer's protocol. Primers used for aromatase (Cyp19a1), GR and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were as follows: Cyp19a1 F – GCCCTTCTTTATGAAAGCTC, Cyp19a1 R – AGGCGTTAAAGTAACCCTGGA, GR F – TGCTATGCTTTGCTCCTGATCTG, GR R – TGTCAGTTGATAAAACCGCTGCC, GAPDH F – ACCACAGTCCATGCCATCAC, GAPDH R – TCCACCACCCTGTTGCTGTA. qPCR reactions were run in triplicate. The threshold cycle (Ct) is defined as the fractional cycle number at which the fluorescence passes a fixed threshold. Relative mRNA units were calculated from these Ct values as described previously (Yilmaz, Wolfe et al. 2009). RNA samples were normalized to GAPDH, an endogenous control. Template-free and RT-negative controls were used to ensure reaction specificity and the absence of genomic DNA. A minimum of 3 independent experiments were carried out to ensure reproducibility.

2.3 Protein Isolation and Immunoblotting

Protein was isolated from neuronal cell lines using mammalian protein extraction reagent (mPER, Thermo Fisher Scientific, Rockford, IL) according to the manufacturer's instruction. Immunoblotting was performed with 25 µg protein extract using standard procedures. Antibodies used were anti-glucocorticoid receptor (Santa Cruz Biotechnology, Santa Cruz, CA, sc-1002), anti-aromatase (Santa Cruz Biotechnology, sc-14245) and anti-β-actin (Sigma-Aldrich, A1978). Quantification of proteins was performed using ImageJ software provided by the NIH and normalized to β-actin.

2.4 Aromatase Promoter Reporter Assays

Transient transfection of cell lines using Fugene HD transfection reagent (Roche Diagnostics, Pleasanton, CA) was performed using 1 µg of pGL3-basic luciferase reporter plasmid containing various lengths of the mouse aromatase promoter I.f/Ebrain (−1000/−1, −700/−1, −500/−1, −200/−1) and 50 ng of pRL-TK plasmid (Promega, Madison, WI) as an internal control. 24 hrs after transfection, cell lines were treated with dexamethasone. At the end of the treatment period, cells were washed with PBS and lysed in 250 µl lysis buffer (0.1 M potassium phosphate [pH 7.8], 1% Triton X-100, 1 mM dithiothreitol and 2 mM EDTA). The Dual-Luciferase Reporter Assay System (Promega) was used to measure luciferase intensity on a LUMAT LB9507 luminometer (EG&G Berthold, Bad Wildbad, Germany). Results are reported as a ratio to the expression of the internal Renilla luciferase standard.

2.5 Glucocorticoid Receptor Knockdown

Small-interfering RNAs (siRNAs) were purchased from Dharmacon (Chicago, IL). GR knockdown was verified by qPCR and immunoblotting. Transfection of 100 nM non-specific siRNA or GR siRNA was performed in triplicate using lipofectamide RNAiMAX reagent (Invitrogen Life Technologies Inc., New York NY) according to the manufacturer's protocol. 48 hrs post-transfection, cells were serum starved for 18 hrs before dexamethasone treatment.

2.6 Chromatin Immunoprecipitation

Chromatin Immunoprecipitation (ChIP) assays were performed using a modified protocol described by Lee et al (Lee, Johnstone et al. 2006). Following a 30-min dexamethasone treatment in 37% formaldehyde, 10⁸ cells for each treatment condition were fixed and sonicated for 22 cycles. Dynabeads (Invitrogen) were conjugated to GR antibody (Santa Cruz Biotechnology) or IgG (Sigma-Aldrich) as a control. Following DNA purification, 1 µL of each IP or input was used in a qPCR reaction using primers across promoter I.f of the Cyp19a1 gene. Quantification was determined as fold enrichment compared to IgG and normalized to vehicle treatment. A minimum of 3 independent experiments were performed to ensure reproducibility.

2.7 Statistical Analysis

All experiments were run independently in triplicate to ensure reproducibility. Statistical significance was determined using either student's t-test or two-way ANOVA followed by Tukey post-hoc test. Error bars represent standard error or means (SEMs) from 3 experiments. Statistical significance was determined at p<0.05.

3. Results

3.1 Effects of the glucocorticoid dexamethasone on aromatase expression in hypothalamic neuronal cell lines

In order to determine the effects of glucocorticoids on neuronal aromatase expression we treated hypothalamic cell lines with the glucocorticoid dexamethasone. We observed similar and comparable effects of glucocorticoids on aromatase expression in four different mouse hypothalamic cell lines (N1, N37, N38 and N42; data for N1, N37 and N38 not shown). We have presented data only from cell line N42 because it showed the most robust glucocorticoid effect. Hypothalamic neuronal cells (N42) were treated with increasing doses of dexamethasone ranging from 10^{-12} to 10^{-4} M for 24 hrs (Figure 1A). Aromatase expression was measured using qPCR. A dose-dependent induction of Cyp19a1 (aromatase) mRNA was observed beginning at 10^{-9} M ($p < 0.05$) and reached a peak of 60-fold induction at 10^{-6} M ($p < 0.05$). We then treated N42 cells with 10^{-7} M dexamethasone over a time period of 15 minutes to 2 days (Figure 1B). A robust 8-fold induction of aromatase was observed as early as 2 hrs ($p < 0.05$) and increased to a maximal 80- to 100-fold induction at 2 days ($p < 0.05$). A robust increase in aromatase protein levels was observed following 24-hr or 48-hr dexamethasone treatment (Figure 1C). There was a significant increase in aromatase levels at 48 hours compared to all other time periods, except at 24 hours (Figure 1D; $p < 0.05$). These data demonstrate the glucocorticoid dexamethasone induced aromatase expression in a time- and dose-dependent manner. This glucocorticoid-dependent and statistically significant aromatase induction was also observed in N1, N37 and N38 cells (data not shown), indicating that this effect is reproducible across murine hypothalamic cell lines.

3.2 Glucocorticoid-dependent induction of the brain-specific aromatase promoter

Using a luciferase reporter assay, dexamethasone activated the distal brain-specific aromatase promoter I.f in the hypothalamic neuronal cell lines N38 (data not shown) and N42 (Figure 2). We generated a series of truncated reporter constructs within the – 1000/–1 region of promoter I.f and transiently transfected them into hypothalamic cells. Dexamethasone robustly induced activity of the larger I.f promoter constructs (–1000/–1, –700/–1 and –500/–1, $p < 0.05$) compared with the empty luciferase vector (Figure 2). This large induction was not observed with the shorter construct (–200/–1). These data indicate that dexamethasone induces aromatase expression, and suggest that the key regulatory elements necessary for this induction lie between 200 and 500 bp upstream of the transcription start site of promoter I.f.

3.3 Glucocorticoid receptor (GR) mediates dexamethasone-dependent induction of aromatase

In order to determine the mechanism of glucocorticoid-induced aromatase expression, GR expression or its activity were chemically and transcriptionally ablated from hypothalamic cell lines. Dexamethasone induction of aromatase was abolished following administration of the GR inhibitor RU-486. N42 cells were treated with dexamethasone (10^{-7} M) in the presence or absence of inhibitor compound (Figure 3A). After 6 hrs, dexamethasone induced aromatase mRNA by 10-fold in the absence of an inhibitor ($p < 0.05$), whereas the addition of RU-486 completely abolished aromatase induction ($p < 0.05$). On the other hand, inhibition of mineralocorticoid receptor by the aldosterone antagonist spironolactone (10^{-5} M) does not alter dexamethasone-dependent aromatase induction. Furthermore, knock-down of GR expression by siRNA (Figure 3B and 3C) abolished dexamethasone-induced aromatase expression (Figure 3D, $p < 0.05$). These data indicate that dexamethasone-induced expression of aromatase in N42 cells is GR-dependent. These findings were replicated in a second hypothalamic cell line, N38 (data not shown).

3.4 Dexamethasone enhances GR recruitment to the brain-specific aromatase promoter I.f

To demonstrate dexamethasone-dependent GR recruitment to the brain-specific aromatase promoter I.f, ChIP was performed in N42 cells following dexamethasone (10^{-7} M) or vehicle (ethanol) treatment. Based on data from our I.f promoter activity reporter assays (Figure 2) and the presence of glucocorticoid-response element half-sites at -310, -460 and -480 bp in the I.f promoter, we tested whether GR binds to the I.f promoter regulatory region that confers dexamethasone induction. When we used two primer sets spanning regions -309 bp to -109 bp (Figure 4B) or -537 bp to -286 bp (Figure 4C) upstream of the transcriptional start site of promoter I.f, there was significant enrichment of GR binding following dexamethasone treatment ($p < 0.05$). Dexamethasone-dependent enrichment of GR binding was not observed at more distal regions of promoter I.f (-1203 bp to -978 bp, Figure 4D). We concluded that dexamethasone-dependent GR recruitment was specific only for the -309/-109 bp and -537/-286 bp regions, which overlap the region that conferred dexamethasone-dependent promoter activity in our promoter-activity reporter assay (Figure 2). Conversely, the dexamethasone effect could not be demonstrated at -1203/-978 bp or at least four other non-GR targeted regions, including the coding exon 8 of the *Cyp19a1* gene (data not shown). These data strongly suggest that GR is recruited to a critical region upstream of aromatase promoter I.f in a glucocorticoid-dependent manner. Significantly, the critical regulatory regions that we identified independently by luciferase reporter assay and ChIP assay overlap, suggesting that glucocorticoid-induction of aromatase in N42 hypothalamic cells is mediated through the brain-specific promoter I.f in a GR-dependent manner.

4. Discussion

In the present study, we demonstrated that glucocorticoids induce aromatase expression via binding of GR to the proximal region of the brain-specific aromatase promoter I.f in the hypothalamus. Aromatase catalyzes conversion of the C19 steroids androstenedione and testosterone to the estrogens estrone and estradiol, respectively (Simpson and Davis 2001; Bulun, Lin et al. 2005). Estrone is weakly estrogenic, whereas estradiol (E_2) has full biologic activity. A number of enzymes present in many tissues can convert estrone to E_2 . Aromatase is encoded by the human gene *CYP19A1A*. Aromatase expression is controlled by ten currently known tissue-specific promoters distributed over a 93-kilobase regulatory region (Sebastian and Bulun 2001; Bulun, Takayama et al. 2004). This regulatory region is upstream of a 30-kb common coding region in chromosome 15 (Chen, Besman et al. 1988). A distinct set of transcription factors regulates each promoter in a signaling pathway- and tissue-specific manner.

The mouse aromatase gene (*Cyp19a1*) has a similar structure to its human counterpart, is located on chromosome 9 and spans nearly 105 kb in total length (Golovine, Schwerin et al. 2003). This includes a 30-kb coding region and an estimated 75-kb regulatory region (Zhao, Innes et al. 2009). This regulatory region contains four promoters that direct transcription of alternatively used untranslated first exons onto a common splice junction that is 15 bp upstream of the ATG translation start site in the coding exon II. This results in multiple aromatase mRNAs with unique 5'-UTRs but an identical coding region. To date, four promoters have been identified: one gonadal promoter that regulates aromatase expression both in the ovary and testis, one testicular promoter active only in the testis, one adipose-specific promoter that regulates aromatase expression in the male gonadal fat pad, and one brain-specific promoter (Zhao, Innes et al. 2009). The physiological importance of individual promoters is not known. The brain-specific aromatase promoter region is highly conserved across vertebrates. There is 93.4% homology between humans and mice in the first 1 kb of the I.f promoter region. This study utilized embryonic-derived murine hypothalamic cells. This model may reflect the mechanisms pertaining to the human

promoter I.f regulation due to the high level of genomic conservation, however there is always the potential for differences between murine and human models. Similarly, these cells are derived from embryos, and could potentially be governed by different mechanisms than adults.

In the present study, we show that the induction of aromatase in the brain by glucocorticoids involves the brain-specific promoter. This mechanism is distinct from the effect of glucocorticoids in peripheral tissues (vide infra). Using ChIP, we also demonstrated that this response involves GR binding to proximal regions of the brain-specific I.f promoter.

In vertebrates, aromatase is expressed in neurons of the hypothalamus, hippocampus and amygdala via the highly conserved promoter I.f (Lephart 1997; Honda, Harada et al. 1999). In the hypothalamus, aromatase is expressed primarily in the preoptic nucleus and the ventromedial nucleus of the hypothalamus (VMH) which are the centers that govern reproductive and neuroendocrine functions of both sexes (Lephart 1996; Peterson, Yarram et al. 2005; Voigt, Ball et al. 2007; Zhao, Fujinaga et al. 2007). An increase in aromatase in the hypothalamus is likely to result in dysregulation of gonadotropin secretion and could account for anovulation in the setting of elevated glucocorticoids.

Pioneering studies investigating the effects of glucocorticoids on aromatase in peripheral tissues showed a similar dexamethasone-dependent induction (Zhao, Nichols et al. 1995). These studies were performed in tissues that do not utilize the brain-specific promoter I.f, however (Agarwal, Bulun et al. 1996; Imir, Lin et al. 2007; Ishikawa, Fenkci et al. 2008; Chen, Reierstad et al. 2011). Interestingly, fetal bovine serum enhanced aromatase expression via promoter I.4, and specifically, cytokines such as IL-11 and activation of Jak2/Stat3 were implicated in the mechanism underlying this effect of serum (Zhao, Nichols et al. 1995). In hypothalamic cells, serum inhibited dexamethasone-dependent induction of aromatase via promoter I.f. We investigated whether a number of class I cytokines such as IL-11 in serum could be responsible for glucocorticoid inhibition (data not shown); but none of these off-the-shelf substances mimicked the serum effects. Thus, the specific substance in serum required for inhibition of glucocorticoid-dependent I.f induction still remains unknown.

Previous studies showed that GR regulated the interaction between promoter I.4 and glucocorticoids. The main glucocorticoid secreted physiologically, cortisol, exhibits both glucocorticoid and mineralocorticoid activity. The GR agonist used in the current study, dexamethasone, is much more specific to GR activity than MR activity. Dexamethasone has nearly 30-fold increased GR activity compared to cortisol, and very little MR activity (Stewart 2008). Furthermore, inhibition of MR activity with the antagonist spironolactone did not inhibit dexamethasone-mediated aromatase upregulation. These data agree with previous studies investigating the role of glucocorticoid activity regulating aromatase.

Previously, we demonstrated that progesterone inhibits aromatase in the hypothalamus in a progesterone receptor (PR)-dependent manner at the -800/-600 bp region of promoter I.f (Yilmaz, Wolfe et al. 2011). This inverse regulation of aromatase by PR and GR is interesting considering that classic GR response elements have the same sequence as PR response elements (Jantzen, Strahle et al. 1987; Strahle, Klock et al. 1987). However, we were unable to identify any classic palindromic GR response elements in the I.f promoter. We did observe 3 half-sites as well as other cis-regulatory element binding sites, such as Sp1, which could serve as docking sites for GR. Furthermore, recruitment of co-activators and co-repressors is likely different between GR and PR, which could explain the difference in aromatase regulation, we observed in response to dexamethasone and progesterone. In this study, we used RU-486 as a GR antagonist; interestingly, RU-486 was originally

identified as a PR antagonist (Cherfas 1989). RU-486 exhibits primarily antiglucocorticoid and antiprogesterin activities. Its binding affinity for GR is 10-fold higher than cortisol and 4-fold higher than dexamethasone, while its binding affinity for PR is 2.5-fold higher than progesterone (Heikinheimo, Kekkonen et al. 2003). The nonspecific nature of RU-486 makes the interpretation of its direct effects challenging; however, the opposing effects of progestins and glucocorticoids in this cell line along with the concordance between RU-486 results and GR-knockdown indicate that in this setting, its primary effect is through inhibition of GR. Testosterone and estradiol have been shown to alter aromatase expression and activity in both mammals and birds. Androgen-dependent aromatase regulation involves a synergism of AR and ER signaling. Interestingly, there are distinct populations of cells that express aromatase but lack AR or ER expression (Balthazart, Baillien et al. 2003). Sex steroid-dependent aromatase regulation in these cells appears to be controlled by inputs from surrounding cells in a manner similar to regulation of GnRH neurons (Absil, Baillien et al. 2001). This mechanism differs from our findings for glucocorticoid-regulated aromatase, as GR is expressed in aromatase containing neurons.

One limitation of our paper is the lack of *in vivo* animal data. Although the presented hypothalamic cell-based data correlate with human conditions associated with CRH-glucocorticoid excess, such as hypothalamic anovulation, there are no useful animal models for this condition. The *in vivo* representation of our findings will require complex models that test the local effects of glucocorticoid on aromatase specific brain regions, and thus is beyond the scope of this study.

In summary, we demonstrated a unique interaction at the transcriptional level between glucocorticoids and aromatase expression in hypothalamic neuronal cell lines. The regulation of aromatase in the brain by GR may partially explain glucocorticoid-dependent disruption of menstruation that is observed in disorders such as Cushing's syndrome or hypothalamic anovulation associated with stress-related cortisol excess. Further work using relevant *in vivo* models is required to confirm our observations.

Acknowledgments

Support

These studies were supported by an ARRA supplement to the U54 Grant HD040093.

References

- Absil P, Baillien M, et al. The control of preoptic aromatase activity by afferent inputs in Japanese quail. *Brain Res Brain Res Rev.* 2001; 37(1-3):38-58. [PubMed: 11744073]
- Agarwal VR, Bulun SE, et al. Use of alternative promoters to express the aromatase cytochrome P450 (CYP19) gene in breast adipose tissues of cancer-free and breast cancer patients. *J Clin Endocrinol Metab.* 1996; 81(11):3843-3849. [PubMed: 8923826]
- Balthazart J, Baillien M, et al. Multiple mechanisms control brain aromatase activity at the genomic and non-genomic level. *J Steroid Biochem Mol Biol.* 2003; 86(3-5):367-379. [PubMed: 14623533]
- Bulun SE, Lin Z, et al. Regulation of aromatase expression in estrogen-responsive breast and uterine disease: from bench to treatment. *Pharmacol Rev.* 2005; 57(3):359-383. [PubMed: 16109840]
- Bulun SE, Takayama K, et al. Organization of the human aromatase p450 (CYP19) gene. *Semin Reprod Med.* 2004; 22(1):5-9. [PubMed: 15083376]
- Chen D, Reierstad S, et al. JunD and JunB integrate prostaglandin E2 activation of breast cancer-associated proximal aromatase promoters. *Mol Endocrinol.* 2011; 25(5):767-775. [PubMed: 21393445]
- Chen SA, Besman MJ, et al. Human aromatase: cDNA cloning, Southern blot analysis, and assignment of the gene to chromosome 15. *DNA.* 1988; 7(1):27-38. [PubMed: 3390233]

- Cherfas J. Dispute surfaces over paternity of RU 486. *Science*. 1989; 246(4933):994. [PubMed: 2587988]
- Golovine K, Schwerin M, et al. Three different promoters control expression of the aromatase cytochrome p450 gene (cyp19) in mouse gonads and brain. *Biol Reprod*. 2003; 68(3):978–984. [PubMed: 12604651]
- Heikinheimo O, Kekkonen R, et al. The pharmacokinetics of mifepristone in humans reveal insights into differential mechanisms of antiprogesterone action. *Contraception*. 2003; 68(6):421–426. [PubMed: 14698071]
- Honda S, Harada N, et al. Identification of cis-acting elements in the proximal promoter region for brain-specific exon 1 of the mouse aromatase gene. *Brain Res Mol Brain Res*. 1999; 66(1–2):122–132. [PubMed: 10095084]
- Honda S, Harada N, et al. The alternative exons 1 of the mouse aromatase cytochrome P-450 gene. *Biochim Biophys Acta*. 1996; 1305(3):145–150. [PubMed: 8597600]
- Imir AG, Lin Z, et al. Aromatase expression in uterine leiomyomata is regulated primarily by proximal promoters I.3/II. *J Clin Endocrinol Metab*. 2007; 92(5):1979–1982. [PubMed: 17341559]
- Ishikawa H, Fenkci V, et al. CCAAT/enhancer binding protein beta regulates aromatase expression via multiple and novel cis-regulatory sequences in uterine leiomyoma. *J Clin Endocrinol Metab*. 2008; 93(3):981–991. [PubMed: 18182446]
- Jantzen HM, Strahle U, et al. Cooperativity of glucocorticoid response elements located far upstream of the tyrosine aminotransferase gene. *Cell*. 1987; 49(1):29–38. [PubMed: 2881624]
- Kalantaridou SN, Makriganakis A, et al. Stress and the female reproductive system. *J Reprod Immunol*. 2004; 62(1–2):61–68. [PubMed: 15288182]
- Lee TI, Johnstone SE, et al. Chromatin immunoprecipitation and microarray-based analysis of protein location. *Nat Protoc*. 2006; 1(2):729–748. [PubMed: 17406303]
- Lephart ED. A review of brain aromatase cytochrome P450. *Brain Res Brain Res Rev*. 1996; 22(1):1–26. [PubMed: 8871783]
- Lephart ED. Molecular aspects of brain aromatase cytochrome P450. *J Steroid Biochem Mol Biol*. 1997; 61(3–6):375–380. [PubMed: 9365213]
- Magiakou MA, Mastorakos G, et al. The hypothalamic-pituitary-adrenal axis and the female reproductive system. *Ann N Y Acad Sci*. 1997; 816:42–56. [PubMed: 9238254]
- Peterson RS, Yarram L, et al. Aromatase is pre- synaptic and sexually dimorphic in the adult zebra finch brain. *Proc Biol Sci*. 2005; 272(1576):2089–2096. [PubMed: 16191621]
- Rivier C, Vale W. Influence of corticotropin-releasing factor on reproductive functions in the rat. *Endocrinology*. 1984; 114(3):914–921. [PubMed: 6321146]
- Sebastian S, Bulun SE. A highly complex organization of the regulatory region of the human CYP19 (aromatase) gene revealed by the Human Genome Project. *J Clin Endocrinol Metab*. 2001; 86(10):4600–4602. [PubMed: 11600509]
- Simpson ER, Davis SR. Minireview: aromatase and the regulation of estrogen biosynthesis--some new perspectives. *Endocrinology*. 2001; 142(11):4589–4594. [PubMed: 11606422]
- Stewart, PM. The Adrenal Cortex. In: Kronenberg, H.; Williams, RH., editors. *Williams textbook of endocrinology*. Philadelphia, Saunders/Elsevier: 2008. p. 445-504.
- Strahle U, Klock G, et al. A DNA sequence of 15 base pairs is sufficient to mediate both glucocorticoid and progesterone induction of gene expression. *Proc Natl Acad Sci U S A*. 1987; 84(22):7871–7875. [PubMed: 2891134]
- Voigt C, Ball GF, et al. Neuroanatomical specificity of sex differences in expression of aromatase mRNA in the quail brain. *J Chem Neuroanat*. 2007; 33(2):75–86. [PubMed: 17270396]
- Xiao E, Luckhaus J, et al. Acute inhibition of gonadotropin secretion by corticotropin-releasing hormone in the primate: are the adrenal glands involved? *Endocrinology*. 1989; 124(4):1632–1637. [PubMed: 2494035]
- Yilmaz MB, Wolfe A, et al. Aromatase promoter I.f is regulated by estrogen receptor alpha (ESR1) in mouse hypothalamic neuronal cell lines. *Biol Reprod*. 2009; 81(5):956–965. [PubMed: 19605792]
- Yilmaz MB, Wolfe A, et al. Aromatase promoter I.f is regulated by progesterone receptor in mouse hypothalamic neuronal cell lines. *J Mol Endocrinol*. 2011; 47(1):69–80. [PubMed: 21628418]

- Zhao C, Fujinaga R, et al. Region-specific expression and sex-steroidal regulation on aromatase and its mRNA in the male rat brain: immunohistochemical and in situ hybridization analyses. *J Comp Neurol.* 2007; 500(3):557–573. [PubMed: 17120292]
- Zhao H, Innes J, et al. A novel promoter controls Cyp19a1 gene expression in mouse adipose tissue. *Reprod Biol Endocrinol.* 2009; 7:37. [PubMed: 19393092]
- Zhao Y, Nichols JE, et al. Aromatase P450 gene expression in human adipose tissue. Role of a Jak/STAT pathway in regulation of the adipose-specific promoter. *J Biol Chem.* 1995; 270(27):16449–16457. [PubMed: 7608217]

Figure 1.

Dexamethasone induces aromatase (Cyp19a1) expression in a murine hypothalamic neuronal cell line (N42) in a dose- and time-dependent manner. A) N42 cells were treated for 24 hrs with escalating doses of dexamethasone, ranging from 10^{-12} to 10^{-4} M. qPCR showed a robust increase in aromatase mRNA expression starting at 10^{-9} M ($p < 0.05$) and peaking at 10^{-6} M ($p < 0.05$). Error bars represent SEMs derived from 3 independent experiments. B) N42 cells were treated with dexamethasone (10^{-7} M) over time periods of 15 min to 48 hrs. Upregulation of aromatase mRNA was observed by qPCR beginning at 2 hrs ($p < 0.05$) post treatment and increased until peaking at 12 hrs and all other later time periods ($p < 0.05$). Error bars represent SEMs derived from 3 independent experiments. C) Increased aromatase protein expression was observed at 24 hrs and 48 hrs following treatment (10^{-7} M) by immunoblotting. Representative immunoblot of 3 independent experiments is shown. D) Quantification of aromatase protein levels was performed using ImageJ software. A statistically significant increase ($p < 0.05$) was observed at 48 hrs following two-way ANOVA followed by Tukey post-hoc test.

Figure 2.

Dexamethasone activates the brain-specific aromatase promoter I.f. A series of truncated murine aromatase promoter I.f regions was inserted into luciferase plasmids. The reporter plasmids were transfected into hypothalamic cell line N42 and the cells were treated with dexamethasone. Promoter activation was measured as increased luciferase activity/luminescence and reported relative to a Renilla standard to control for transfection efficiency. Increased luciferase activity was observed following dexamethasone (10^{-7} M) treatment in larger promoter region constructs (–1000, –700 and –500; $p < .05$) but not in shorter constructs (–200). Error bars represent SEMs derived from 3 independent experiments.

Figure 3.

Dexamethasone induction of aromatase requires GR. A) Hypothalamic cell line N42 was treated with dexamethasone (10^{-7} M) for 24 hrs in the presence or absence of GR inhibitor RU-486 (10^{-5} M), or in the presence or absence of MR inhibitor spironolactone (10^{-5} M). Aromatase expression was measured by qPCR. Error bars represent SEMs derived from 3 independent experiments. RU-486 inhibited dexamethasone-induced aromatase upregulation but spironolactone did not have an effect on aromatase expression ($p<.05$) B) N42 hypothalamic cells were transfected with siRNA targeted to GR or a scrambled siRNA control. GR siRNA successfully decreased GR mRNA expression as observed by qPCR ($p<.05$). Error bars represent SEMs derived from 3 independent experiments. C) siRNA successfully decreased GR protein expression as observed by immunoblotting. D) Hypothalamic cell lines transfected with GR-siRNA were treated with dexamethasone (10^{-7} M) for 24 hrs. Knockdown of GR abolished dexamethasone-induced aromatase expression in these cells ($p<.05$). Error bars represent SEMs derived from 3 independent experiments.

Figure 4.

GR binds to aromatase promoter I.f in hypothalamic neuronal cell line N42. A) Schematic representation of the aromatase I.f promoter region. The location of ChIP primers is indicated as well as the candidate response element binding sites (non-palindromic GR half-sites and potential docking sites, Sp1). ChIP was performed on hypothalamic cell lines treated with dexamethasone (10^{-7} M) or vehicle (ethanol). Following dexamethasone treatment, recruitment of GR was observed at proximal regions of the brain-specific aromatase promoter from (B) -309 bp to -109 bp and (C) -537 bp to -286 bp). Data are reported as fold enrichment compared to IgG. Error bars represent SEMs derived from 3 independent experiments. D) GR recruitment was not observed at more distal regions (-1203 bp to -978 bp) of aromatase promoter I.f, further indicating that the GR enrichment at the proximal promoter region is specific.