Steroids 107 (2016) 128–138

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Regulation of gene expression by 17β-estradiol in the arcuate nucleus of the mouse through ERE-dependent and ERE-independent mechanisms

Jennifer A. Yang, Kyle J. Mamounis, Ali Yasrebi, Troy A. Roepke *

Department of Animal Sciences, School of Environmental and Biological Sciences, Rutgers University, New Brunswick, NJ, USA

ARTICLE INFO

Article history:
Received 11 July 2015
Received in revised form 29 December 2015
Accepted 3 January 2016
Available online 6 January 2016

Keywords:
17β-Estradiol
Ovariectomy
ERα
Arcuate nucleus

ABSTRACT

17β-Estradiol (E2) modulates gene expression in the hypothalamic arcuate nucleus (ARC) to control homeostatic functions. In the ARC, estrogen receptor (ER) α is highly expressed and is an important contributor to E2's actions, controlling gene expression through estrogen response element (ERE)-dependent and -independent mechanisms. The objective of this study was to determine if known E2-regulated genes are regulated through these mechanisms. The selected genes have been shown to regulate homeostasis and have been separated into three subsections: channels, receptors, and neuropeptides. To determine if ERE-dependent or ERE-independent mechanisms regulate gene expression, two transgenic mouse models, an ERα knock-out (ERKO) and an ERα knock-in/knock-out (KIKO), which lacks a functional ERE binding domain, were used in addition to their wild-type littermates. Females of all genotypes were ovariectomized and injected with oil or estradiol benzoate (E2B). Our results suggest that E2B regulates multiple genes through these mechanisms. Of note, *Cacna1g* and *Kcnmb1* channel expression was increased by E2B in WT females only, suggesting an ERE-dependent regulation. Furthermore, the NKB receptor, *Tac3r*, was suppressed by E2B in WT and KIKO females but not ERKO females, suggesting that ERα-dependent, ERE-independent signaling is necessary for *Tac3r* regulation. The adrenergic receptor *Adra1b* was suppressed by E2B in all genotypes indicating that ERα is not the primary receptor for E2B's actions. The neuropeptide *Tac2* was suppressed by E2B through ERE-dependent mechanisms. These results indicate that E2B activates both ERα-dependent and independent signaling in the ARC through ERE-dependent and ERE-independent mechanisms to control gene expression.

1. Introduction

The steroid hormone 17β-estradiol (E2) is known to regulate gene expression throughout the brain. E2 primarily uses two classical nuclear receptors, estrogen receptor α (ERα, *Esr1*), and ERβ (*Esr2*) to regulate gene expression [1]. In the classical ER-mediated mechanism, ligand binding to the receptor initiates receptor recognition of the estrogen response element (ERE) to regulate gene transcription. In addition to classical regulation of gene expression, E2 also functions through ERE-independent mechanisms. As reviewed in McDevitt et al., these mechanisms include ligand-independent ER signaling, rapid effects through a membrane-associated ER, and ERE-independent signaling through protein-protein interactions (AP-1, etc.) [2].

In the hypothalamus, E2 mediates numerous homeostatic functions including reproduction, energy homeostasis, core body temperature, fluid balance, motivational behaviors, and stress physiology by regulating central neural pathways. Many of these pathways originate in or pass through the arcuate nucleus (ARC)

Abbreviations: *Abcc8*, ATP-binding cassette, subfamily C; *Actb*, beta actin; *Adra1b*, alpha-1B adrenergic receptor; AgRP, agouti-related protein; ARC, arcuate nucleus; AVPV, anteroventral periventricular nucleus; *Bcl2*, B cell leukemia/lymphoma 2; BH, basal hypothalamus; *Cacna1g*, T-type, voltage-dependent, calcium channel alpha 1G subunit; *Cart*, cocaine- and amphetamine-regulated transcript; *Chrm1*, cholinergic muscarinic 1 receptor; E2, 17β-estradiol; E2B, 17β-estradiol benzoate; ER, estrogen receptor; ERE, estrogen response element; ERKO, ERα knock-out; ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; *Esr1*, estrogen receptor alpha; *Esr2*, estrogen receptor beta; *Gapdh*, glyceraldehyde-3-phosphate dehydrogenase; *Ghsr*, growth hormone secretagogue receptor; GnRH, gonadotropin releasing hormone; GPCR, G-protein coupled receptor; GPER, G protein-coupled estrogen receptor 1; HPG, hypothalamic–pituitary–gonadal; HRE, hormone response element; *Kcnmb1*, calcium-activated potassium channel subunit β1; KIKO, knock-in/knock-out; *Kiss1*, kisspeptin; *Kiss1r*, kisspeptin receptor; KNDy, kisspeptin–neurokinin B–dynorphin; Mtor, mammalian target of rapamycin; NPY, neuropeptide Y; ovx, ovariectomized; *Pdyn*, prodynorphin; *Pgr*, progesterone receptor; POMC, proopiomelanocortin; qPCR, quantitative real-time polymerase chain reaction; *Tac2*, tachykinin 2; *Tac3r*, tachykinin 3 receptor; TH, tyrosine hydroxylase; TLDA, Taqman Low Density Array; WT, wild-type.

* Corresponding author at: Department of Animal Sciences, School of Environmental and Biological Sciences, Rutgers University, 84 Lipman Drive, New Brunswick, NJ 08901, USA.

E-mail address: ta.roepke@rutgers.edu (T.A. Roepke).

http://dx.doi.org/10.1016/j.steroids.2016.01.003
0039-128X/

of the hypothalamus [1,3–5]. In the ARC, ERα is highly expressed and is the primary receptor used by E2 to control many homeostatic functions [5,6]. Few studies have examined the physiological effects of ERα-mediated, ERE-dependent and ERE-independent signaling on hypothalamic (ARC) gene expression.

Recently, the development of an ERα knock-in/knock-out (KIKO) mouse model lacking a functional DNA-binding domain (no ERE binding) gives insight to nonclassical, ERα-mediated, ERE-independent signaling while retaining ERβ-mediated signaling and other extra-nuclear initiated pathways (GPER, Gq-mER) [7,8]. While KIKO females are infertile due to an absence of the LH surge [9,10], they exhibit similar body weights, feeding, activity, oxygen consumption, glucose homeostasis, and hypothalamic leptin sensitivity compared to wild-type (WT) females, unlike their total ERKO counterparts [11]. However, recent data from our lab suggest that ERα-mediated, ERE-independent signaling partially restores the post-ovariectomy (ovx) weight gain but is not sufficient to mediate E2's attenuation of this weight gain [12].

E2 control of homeostatic functions occurs, in part, through regulation of important genes in the ARC. However, only a few studies have examined which signaling mechanisms E2 utilizes to control the expression of these genes. The KIKO mouse model provides an appropriate tool to increase our understanding of how homeostatic genes are regulated by E2-induced, ERα-mediated mechanisms in the ARC. In the ARC, E2 is known to regulate the expression of a variety of cation channels including calcium channels and potassium channels [13–16]. The expression and activity of these cation channels are involved in regulating ARC proopiomelanocortin (POMC) and neuropeptide Y (NPY) neurons and their neuronal excitability [1,12,14–20]. Furthermore, E2 is known to regulate the mRNA expression of signaling molecules such as calmodulin and phosphatidylinositol-4,5-biphosphate 3-kinase (PI3K) [16,21–23] and neurotransmitter enzymes like tyrosine hydroxylase (TH) and glutamate decarboxylase [1,24]. Many of these E2-regulated genes are involved in reproduction, energy homeostasis, and hormone receptor signaling [1,3,25–27].

E2 is also known to suppress or augment the expression of a variety of neuropeptides and receptors in the hypothalamus, depending upon the experimental paradigm. In rodents, E2 increases *Pomc* and suppresses *Npy* expression in the ARC [1] and increases the expression of growth hormone [28]. It is also well known that E2 differentially regulates steroid receptors in the hypothalamus such as ERα, ERβ, and progesterone receptor (*Pgr*) [1,16,29]. Other hypothalamic hormone and neurotransmitter receptors that are regulated by E2 in the hypothalamus include growth hormone secretagogue receptor (*Ghsr*) [30] and serotonin receptor 2C receptor (5HT2c) [31]. The mechanism underlying the regulation of all of these genes is largely unknown. Our current study focused on the regulation of ARC gene expression by E2.

E2 also regulates ARC KNDy (Kisspeptin–Neurokinin B–Dynorphin) neuronal gene expression [32]. Kisspeptin is involved in mediating negative and positive feedback of E2 on the hypothalamic–pituitary–gonadal (HPG) axis and potentially has a role in energy homeostasis and core body temperature [33]. Previous studies indicate that the *Kiss1* gene is regulated by E2 through ERE-independent mechanisms in the mediobasal hypothalamus while dynorphin expression is ERE-dependent [34]. Thus, we used these genes as positive and negative controls for ERE-dependent and ERE-independent gene expression. Nothing is known about the mechanisms behind the regulation of neurokinin B (NKB, *Tac2*) or the KNDy receptors (*Kiss1r*, *Tac3r*) by E2 in the ARC. Therefore, the objective of this study was to determine if E2-responsive, homeostatic genes involved in reproduction and energy homeostasis are regulated by E2 in the ARC through ERE-dependent or ERE-independent mechanisms using ovx WT, KIKO, and ERKO females.

2. Experimental

2.1. Animal care

All animal procedures were in compliance with institutional guidelines based on National Institutes of Health standards and were performed with Institutional Animal Care and Use Committee approval at Rutgers University. Adult C57BL/6 mice were housed under constant photoperiod conditions (12/12 h light/dark cycle) and maintained at a controlled temperature (23 °C). Animals were given low phytoestrogen chow diet (<75 isoflavone ppm, Lab Diet Advanced Protocol 5V75, St. Louis, MO, USA) and water *ad libitum*. Animals were weaned on postnatal day 21 (PD21). Genotype was determined by using PCR products of extracted DNA from ear clippings, using previously published protocols [9]. We used three genotypes of mice: WT, KIKO, and ERKO (provided by Dr. Ken Korach, NIEHS) [9]. Crossing heterozygous WT/KI males expressing the nonclassical ERα knock-in with WT/KO heterozygous females generated WT and KIKO females. Crossing heterozygous WT/KO males and females generated WT and ERKO females. WT females used in the experiments were littermates generated from both colonies.

2.2. Drugs

17β-Estradiol benzoate (E2B) was purchased from Steraloids (Newport, RI, USA) and dissolved in ethanol (1 mg/ml) prior to mixing in sesame oil (Sigma–Aldrich). Ketamine was purchased from Henry Schein Animal Health (Melville, NY, USA) and used for sedation prior to sacrifice.

2.3. Ovariectomy

Adult females (7–22 weeks and >14 g body weight) were bilaterally ovx under isoflurane anesthesia 7 days prior to sacrifice using sterile no-touch techniques according to the NIH "Guidelines for Survival Rodent Surgery." Animals were given a dose of analgesic [4 mg/kg carprofen (Rimadyl®)] one day following surgery for pain management. Animals typically lost 1–2 g of weight one day after surgery. Females were monitored daily and allowed to recover for 5 days prior to the first injection of E2B or oil. The active metabolite of E2B is 17β-estradiol. Females were injected in the morning at 1000 h on post-ovx days 5 and 6 and sacrificed on post-ovx day 7 in the morning at 1000 h.

2.4. Experimental design

Females of each genotype (WT, KIKO, ERKO) were ovx and separated into a control sesame oil-treated group (n = 6–9 per genotype) and an E2B-treated group (n = 6–9 per genotype). An E2B injection protocol was used that has been shown to alter gene expression in the hypothalamus [19]. Animals were injected subcutaneously at 1000 h on post-ovx day 5 with either 0.25 μg of E2B or sesame oil. On post-ovx day 6, a 1.5 μg dose of E2B or sesame oil was injected at 1000 h. We did not include intact females in our experimental design as neither ERKO nor KIKO females exhibit a normal estrous cycle, which makes it difficult to compare among intact WT, KIKO, and ERKO females [11]. Animals were sacrificed on post-ovx day 7 at 1000 h. Animals were sedated with ketamine (100 μl of 100 mg/ml stock, i.p.) and decapitated. Brains were removed and rinsed in ice-cold Sorensen's Phosphate Buffer (0.2 M sodium phosphate, dibasic; and 0.2 M sodium phosphate, monobasic) for 30–60 s. The basal hypothalamus (BH) was cut using a brain slice matrix (Ted Pella, Inc., Redding, CA, USA) into 1-mm thick coronal rostral and caudal blocks corresponding to Plates 42–47 and Plates 48–53, respectively, from