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Contributions of estrogen receptor-α and estrogen receptor-β to the regulation of behavior

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Abstract

Studies of the mechanisms by which estrogens influence brain function and behavior have advanced from the explication of individual hormone receptors, neural circuitry and individual gene expression. Now, we can report patterns of estrogen receptor subtype contributions to patterns of behavior. Moreover, new work demonstrates important contributions of nuclear receptor coactivator expression in the central nervous system. In this paper, our current state of knowledge is reviewed.

Keywords

Estrogen receptor; Hypothalamus; Preoptic area; Lordosis; Aggression; Progestin receptor; Sexual differentiation; Steroid receptor coactivator-1 (SRC-1)

Gene products coding for nuclear hormone receptors have afforded neuroscientists unusual opportunities for the analysis of brain mechanisms that regulate behavior. Brain researchers benefit from the relatively simple chemistry of steroid hormones and from the large numbers of reagents effective in the manipulation of hormone-dependent systems of cells. Success during the recent scientific generations of work in this field has allowed us to understand how sex steroid actions in the brain coordinate behavioral regulation with other factors in the body and in the environment. The upshot is that we understand mechanisms with physical dimensions ranging from Angstrom units (ligand binding domains of estrogen receptors) to light years (the seasonality of reproduction in many animals).

Of particular interest are gene duplication products among steroid hormone receptors, both of which the ancestral hormone can activate both as the normal ligand, but which are genetically and chemically distinguishable. Estrogens elicit many of their effects on behavior and physiology by binding to its intracellular receptors, estrogen receptor- α (ERα) and ERβ, which are transcribed from different genes [1,2] and are members of the steroid/nuclear receptor superfamily of transcriptional activators [3,4]. While estrogens can function in the brain by binding to receptors on the cell membrane and rapidly activate cytoplasmic

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signaling pathways [5–8], this review will focus on the effects of estrogens on behavior and physiology mediated by ERα and ERβ via classic, genomic mechanisms of action.

Using mice created by homologous recombination to achieve null mutations for ERα (ERαKO) or ERβ (ERβKO), we and others have conducted a large number of behavioral and histochemical assays in genetic females and genetic males. Several of the assays simply depend on the classical ER, without a discernible contribution from ERβ. During other assays, expression of ERβ appears to act as a brake on ERα function. In still other assays, ERβ and ERα can substitute for each other.

The contributions of ERα and ERβ to behaviors and physiological events are complicated and in no way uniform. Below we have summarized results gathered to date, and labeled each according to the relative contributions of the ER subtypes. The extreme scenarios are that ERα and ERβ act in an obligatory synergistic fashion {α+β} or that they exert equal and opposite function {α vs. β}. The data suggest that different patterns of neural function require different patterns of contributions by ERα and ERβ, such that: ERα and ERβ can substitute for each other {α or β}; the contributions of ERα and ERβ are not related {α/β contributions nr}; ERβ acts as a brake on ERα function and reduces the effects of ERα {α −β=α′}. While we and others have used many behavioral or histochemical assays, the references below concentrate on data related directly to the brain's regulation of reproduction. Then, our main inference is stated at the end of this paper. For an in depth review of ERα and ERβ expression in brain regions known to regulate behaviors associated with reproduction, including sexual behavior, aggression and anxiety, please see Bodo et al. [9].

1. Behavior

Perhaps the most striking result was the first reported: that putting an ERαKO female into the cage of a sexually experienced male had two surprising results: (i) that she behaves exactly as a male would, with respect to immediate aggressive behavior, and (ii) that she is treated like an intruder male by the resident male [10]. In a broader framework, comparisons among results of the different labs cited below attest to the reliability of neuroendocrine and behavioral results in this field.

1.1. Female sexual behavior {α−β=α′}

Gene expression from ERα raises levels of generalized arousal in female mice [11] eventuating in significantly higher locomotor activity [12], important for the initiation of courtship behaviors. In turn, studies using ERαKO mice and RNA interference indicate that the ERα gene product is essential for the normal performance of lordosis behavior [13–16]. In contrast, while ERβKO females are subfertile [17], their lordosis behavior was equal to that of wt females and somewhat elevated in that they exhibited strong sexual receptivity the day after estrus [14,16]. These results indicate that female sexual behavior is dependent on the classical ERα, and not ERβ which appears to reduce the effects of ERα on sexual receptivity.

1.2. Male sexual behavior {α/β contributions nr}

While adult ERβKO males exhibit normal male sex behavior in regard to mounts, intromissions and ejaculations [16,18], this ER subtype appears to be important for development of male sex behavior. Peripubertal ERβKO males display their first ejaculations at a later age than their wt littermates [19]. In adults, reduced success in progress toward ejaculation is seen in ERαKO males [20]; that is, latencies through the series of intromissions are increased so that the temporal interval from first intromission to eventual ejaculation is increased — ejaculation is delayed. Finally, knocking out both ERα and ERβ completely abolishes male sexual behaviors, including mounting. [21]. Thus it appears that both ER subtypes contribute to the normal display of male sexual behavior. We infer from these data that even under circumstances in which ERβ by itself is not necessary for male sex behavior, it still can add synergistically to ERα, such that the double knockout completely abolishes males sexual behaviors.

1.3. Aggression in male ERKO mice {α−β=α′}

Early work has shown that ERαKO males are less aggressive to a male intruder than wt males [20,22]. Another study found that while wt males treated with testosterone (T) only attack T-treated male intruders, ERαKO males display equal aggression towards T-treated male, and estradiol (E)-treated female, intruders [23]. This inability of ERαKO males to distinguish intruders, possibly due to impaired processing of chemosensory information, suggests that ERα is important in normal social preferences. Furthermore, gonadally intact ERβKO males exhibit higher levels of aggression compared to wt males [20]. In gonadectomized males, estradiol benzoate (EB) treatment induces higher levels of aggression in ERβKO animals than wt [24]. These data suggest that the effects of estrogens on male aggression are stimulated by ERα and suppressed by ERβ.

1.4. Anxiety in females {α−β=α′}

A variety of studies indicate that ovarian hormones influence anxiety in female rodents [25–27]. To test the function of the two ER subtypes in anxiety, ovariectomized rats were given DPN (diarylpropionitrile, an ERβ selective agonist), PPT (Tris(4-hydroxyphenyl)-4-propyl-1H-pyrazole, an ERα selective agonist), E or vehicle and tested for anxiety-related behaviors [28]. DPN treatment decreased anxiety-like behaviors in elevated plus maze and open field tests. Anxiogenic behaviors, including the time spent grooming, were decreased by DPN and increased by PPT [28]. It should be pointed out that the selectivity of DPN and PPT for ERβ and ERα, respectively, are not absolute [29–31]. However, in support of this anxiolytic effect of ERβ, ERβKO females display higher levels of anxiety than wt littermates by spending less time exploring the distal portion of the open arm of a maze [32,33]. This effect in ovariectomized ERβKO mice was observed whether mice were treated with chronic E or vehicle [32]. Taken together, these findings indicate that ERβ is important during development and/or adulthood in mediating the anxiolytic effects of estrogens on anxiety.

1.5. Stress in male rats {α/β contributions nr}

The paraventricular nucleus (PVN) of the hypothalamus is critical in the modulation of stress and expresses high levels of androgen receptors (AR) [34] and ERβ [35–37], but very

little ERα [35]. To examine the role of ERβ and AR in the regulation of the hypothalamic–pituitary–adrenal response to stress, gonadectomized male rats were implanted with pellets containing E, dihydrotestosterone (DHT, which binds AR and is not aromatized to estradiol), 5alpha-androstane-3beta,17beta-diol (3β-diol, which binds ERβ with a higher affinity than ERα; [29], DPN or PPT into the PVN [38]. Following restraint stress, animals treated with DHT, 3β-diol or DPN had reduced levels of stress-induced release of corticosterone and ACTH, while E or PPT treatment increased the release of these stress hormones. These data suggest that ERβ functions to decrease HPA activity, while stimulation of ERα enhances it. The ER antagonist, tamoxifen, partially blocked the effects of 3β-diol and DHT, while an AR antagonist did not. The authors suggest that these effects are mediated through ERβ, since 3β-diol binds ERβ with a higher affinity than ERα [29]. In addition, administration into the PVN of DHT, 3β-diol or DPN reduced the restraint-induced increase in c-*fos* mRNA in this brain region [38]. These findings suggest that ERβ inhibits the HPA axis in males and that DHT may suppress the stress response, in part, via conversion to 3β-diol and its subsequent binding to ERβ.

2. Sexual differentiation of the brain

The gonadal steroids elicit profound organizational effects on the developing brain that result in adult sexually dimorphic brain physiology and behavior. During the perinatal period, the testes secrete testosterone that masculinizes (increased adult male sexual behavior) and defeminizes (decreased adult female sexual behavior) adult reproductive behavior. In the brain, testosterone can be converted to DHT or estradiol, which binds to AR and ER, respectively. Thus, testosterone can ultimately have both androgenic and estrogenic effects on the developing and adult brain.

2.1. Sexual differentiation of dopamine neurons in the anteroventral periventricular area {α or β}

In the hypothalamus of rodents, the anteroventral periventricular area (AVPV) has a sexually dimorphic subpopulation of dopaminergic neurons that is larger in females than males and is thought to be involved in the normal display of the preovulatory surge [39]. Testicular feminized male (tfm) mice, which have disrupted AR, and wt males had equal numbers of tyrosine-hydroxylase (TH)-immunoreactive neurons (indicative of dopamine neurons) in the AVPV, suggesting that AR do not regulate the expression of dopamine neurons in this brain region [40]. However, ERαKO male mice had more TH-immunoreactive neurons than wt males, indicating that disruption of the ERα gene feminized the number of dopaminergic neurons in the AVPV [40]. In an extension of this work, both ERαKO and ERβKO male mice had levels of TH-immunoreactive neurons that were higher than wt males and equal to wt female mice [41]. In addition, treatment of wt female mice during postnatal days 1–3 with either estradiol, DPN or PPT reduced the number of TH-immunoreactive neurons in the AVPV [41]. Taken together, these studies suggest that both ERα and ERβ, and not AR, are important in the sexual differentiation of the dopaminergic system in the AVPV of male and female mice.

3. Estrogen-responsive genes

3.1. Estradiol-induction of PR: females {α|β contributions nr}, males {α+β}, and developing female brain {α−β=α′}

A classic example of an estrogen-responsive gene is the estradiol-induction of PR in a variety of tissues, including brain. While PR are present in low levels in the brains of ovariectomized rodents, E-priming dramatically increases the expression of PR in the medial preoptic area (MPOA), ventromedial hypothalamus (VMH), arcuate and midbrain central gray [42–52]. Interestingly, the developing rat brain is extremely sensitive to estradiol, given that doses as low as 100 ng of E are sufficient to induce PR [53]. In adult female guinea pigs virtually all of the E-induced PR cells express ERα [49,54], while about 30% of the E-induced PR cells in rat brain express ERβ [37].

In females, it appears that ERα is the major contributor to E-induction of PR in the brain. In support, E-induction of PR in the VMH and POA of ERβKO females was equal to wt animals [14,55], suggesting an important role for ERα in these regions. However, while E-induction of PR is greatly diminished in ERαKO female mice, it is not abolished in all areas, including the POA and VMH [14,56,57]. This residual induction of PR by estradiol may be due to ERβ (in the absence of ERα) or ERα splice variants that have been detected in ERαKO mice [56]. In contrast to females, while E treatment did not induce PR expression in the VMH and POA of wt males, it did increase PR levels in both brain regions of ERβKO males [55]. These findings suggest an important role for ERβ in the sexually dimorphic responses to estrogens in reproductively-relevant brain regions. In addition, these data suggest that ERβ can modulate the responsiveness of ERα in some cells.

Emilie Rissman's lab has used heterozygous breeders to generate mice with varying numbers of functional and disrupted ER genes to determine the contribution of each ER subtype to E-induction of PR in the MPOA [58]. Male and female mice with either 0, 1 or 2 functional copies of ERα and/or ERβ were gonadectomized and received implants of estradiol or blank capsules for 5 days. In females, maximal induction of PR by E in the MPOA required at least one functional copy of the ERα gene. In contrast to females, one copy of each ERα and ERβ genes are required for the full induction of PR in the male MPOA. These data support the concept that the role of ERβ in PR induction is sexually dimorphic and that there is functional dependence of the two ERs in PR induction in the male brain.

These findings in the adult brain suggest that ERβ can alter the activity of ERα. This concept is supported by a study in developing rat brain using ER subtype selective agonists [59]. In the developing female ventromedial nucleus of the hypothalamus (VMN), selective activation of ERα with PPT induces PR to higher levels than estradiol alone, which activates both ER subtypes. In contrast, in the MPN, which expresses high levels of ERα and low amounts of ERβ [60], PPT and estradiol induced PR to the same extent. In addition, selective activation of ERβ by DPN did not induce PR in the MPN. Moreover, activation of ERβ diminishes the ERα-mediated increase of PR in the VMN, but not the MPN. These findings suggest that activation of ERβ by estradiol in the VMN inhibits ERα transcriptional activity, and thus suppresses ERα-mediated transactivation of the PR gene in this brain region [59]. This suppressive effect of ERβ may provide a mechanism to protect the VMN

from defeminization by estradiol during important periods of development, thus ensuring proper feminization of the VMN and subsequent adult female sexual behavior. This idea is supported by work in mice showing that activation of ERα or ERβ during development alters female sexual behavior in adulthood [61].

3.2. Estrogen and androgen regulation of AVP {α|β contributions nr}

Arginine vasopressin (AVP), a neurotransmitter involved in aggression, is regulated by androgens and estrogens. Testosterone, and to a lesser extent estradiol, up-regulates AVP expression in brain regions involved in aggression [62,63]. To investigate the roles of ERα and AR in gonadal hormone regulation of AVP and aggression, mice lacking a functional AR (tfm) were compared with ERαKO mice crossed with tfm (resulting in a double AR/ERαKO), ERαKO and wt mice [64]. Gonadectomized mice were treated with estradiol and analyzed by resident-intruder aggression tests. Wt and tfm males displayed aggressive behaviors, while ERαKO males and AR/ERαKO males did not, suggesting that ERα functions in males to regulate aggression as discussed above. AVP levels in the lateral septum revealed that wt and tfm males had the highest levels, followed by ERαKO males, and lowest levels in wt females and AR/ERαKO males. AR/ERαKO males expressed the lowest levels of AVP in the medial amygdala, with all other genotypes expressing equal amounts in this region [64]. In contrast to the lateral septum, estradiol reduces AVP expression in the male PVN [65]. This down-regulation of AVP by estradiol is abolished in ERβKO males, indicating that ERβ is critical for normal AVP expression in this brain region [65]. Interestingly, another study using a neuronal cell line found that DHT, and its metabolite androstane-3β,17β-diol, bind to ERβ to stimulate AVP promoter activity [66]. It will be essential for future studies to more fully investigate the function of ERβ in AVP expression in the brain. Taken together, these findings indicate that both ERα and AR are required for maximal expression of AVP in the lateral septum and medial amygdala, while ERβ is critical in AVP expression in the PVN.

4. ERα and ERβ interactions and mechanisms

In order to better understand the functional differences of ERα and ERβ, one must understand the mechanisms of action of these two subtypes. The ER subtypes have high homology in the DNA binding domain (97%), but much lower homology in the AF-1 and ligand binding domain (60%) [67]. These structural differences allow for variation in the binding affinity and specificity for different ligands of the two ER subtypes [29,68]. In addition, these differences in ligand binding, and the subsequent distinct changes in receptor conformation, enable the ER subtypes to vary in their ability to interact with different estrogen response elements [69]. Thus, ERα and ERβ can have profoundly different effects in the transduction of estrogen signaling.

4.1. ERα and ERβ interactions with nuclear receptor coactivators

In addition to binding to different ligands, ERα and ERβ may elicit distinct transcriptional effects through differential interactions with nuclear receptor coactivators. Nuclear receptor coactivators enhance steroid receptor transcriptional activity *in vitro* [70,71]. Moreover, these coactivators have been found to be critical in estrogen action in brain and behavior

[72,73]. For example, steroid receptor coactivator- 1 (SRC-1), a member of the p160 family of coactivators, is critical for maximal ER-mediated transactivation of the PR gene in the VMN [74,75] and the display of estrogen-dependent behaviors [76]. Recent studies using pull-down assays have explored the physical interactions between the ER subtypes and SRC-1 from female rat brains [77]. SRC-1 from hypothalamus or hippocampus interacted with ERα and ERβ when bound to estradiol, which was confirmed by mass spectrometry. SRC-1 may function with ERα in the hypothalamus to mediate expression of female sexual behavior [13–16], and with both ER subtypes in the hippocampus to differentially modulate estrogen's effects on cognition [9,78] and stress [9,79].

The different functions of the ER subtypes in the brain may be explained in part by the lower transcriptional activity of ERβ observed in particular cell lines [80]. These differences in transcriptional abilities between ERα and ERβ may be attributed to differential recruitment of coactivators, or differences in the ability of the same coactivator to facilitate transcription of the ER subtypes [81]. Interestingly, while SRC-1 from the hippocampus interacted equally with the ER subtypes, SRC-1 from hypothalamus interacted more with ERα than with ERβ. These findings suggest that ERα is a more efficient transcriptional activator of SRC-1 dependent signaling pathways in the hypothalamus than ERβ [77]. In support, previous work indicates that SRC-1 function in the hypothalamus is important for normal expression of ER-mediated female sexual receptivity [76], which as discussed above is ERα-dependent [13–16,82]. These differential interactions of SRC-1 from hypothalamus or hippocampus with the ER subtypes suggest that these brain regions have distinct expression patterns of coregulators involved in these important protein–protein interactions. In addition, it is possible that SRC-1 undergoes differential phosphorylation in these two brain regions, leading to distinct patterns of interaction with the ER subtypes. These findings suggest the importance of using biologically-relevant tissue, in contrast to the use of cell lines alone, in investigating receptor–coactivator interactions. It may be that other cofactors and proteins that are present in tissue, such as the brain, are important for appropriate coactivator interactions with receptor. Understanding how nuclear receptor coactivators function with various steroid receptors, and their subtypes, is critical to understanding how hormones act in different brain regions to profoundly influence physiology and behavior.

It has been suggested that the lack of ERβ results in ERβKO mice that are hypersensitive to E [55]. In support, E-induces elevated levels of PR in the brains of ERβKO males [55] and the uteri of ERβKO females [83]. In addition, sexual behavior and learning studies suggest that ERβKO females are hypersensitive to E [16,78]. ERβ repression of ERα activity may involve the sequestration of shared nuclear receptor coactivators, that is relieved due to the absence of ERβ in ERβKO mice.

4.2. DNA as an allosteric ligand

Another level of ER signaling regulation may be exerted by DNA on the ER subtypes. In a recent elegant series of studies, Meijsing et al. [84] demonstrate that DNA can act as an allosteric ligand of glucocorticoid receptors (GR). Different GR binding sequences, which are imperfect palindromic, hexameric half sites separated by 3-base pair spacers, that differ by as little as one base pair, can elicit distinct conformations in GR and regulate its

transcriptional activity. As suggested by the authors, this idea of DNA sequences acting as allosteric ligands may modulate signaling of other nuclear receptors, including ER [84]. In support, the AF-2 domain of ER binds to different coregulatory peptides when ER is bound to distinct DNA sequences [69,85]. Thus, in addition to the type of ligand that is available to ER, specific DNA sequences that bind the ER subtypes may modulate the conformation of ERα and/or ERβ in the cell and influence the coregulators that it recruits, thus altering the transcriptional activity of the receptors.

5. Conclusions

Taken together, the complex dependencies of results in this field of neuroendocrinology serve to defeat simplistic thinking about gene/behavior relationships. Even with these clear genetic manipulations and straightforward behavioral assays, unexpected results have emerged. For example, with respect to the impact of an ERβKO on aggressive behaviors by male mice, it became clear that the strength of the phenotype depends upon the age at which the male is tested, the KO effect being strongest just after puberty [86]. Even more surprising, the relation between a given gene and a given behavior (in this case, aggressive behavior) can be exactly the opposite between males and females (summarized in [87]). In addition, given the importance of coactivators in steroid receptor signaling, it is important to consider the possible effects on the signaling of one receptor subtype when the other subtype is knocked out. For example, consider a population of cells that coexpress ERα and ERβ [37]. In ERαKO mice, it is possible that in this population of cells, ERβ signaling may be enhanced due to decreased competition for common coactivators now that ERα has been eliminated. While we are not aware of any published data supporting this idea, it would be important to consider and explore these potential cell-specific effects in future studies.

We note that the prize-winning discovery of George Beadle and Edward Tatum, based on their work in 1941 with the fungus *Neurospora*, depended on their enunciation of the “one gene – one enzyme” concept. As distinct from their conclusion, and as inferred in Pfaff et al., 2002 [88], we believe that the strategic advantages of studying steroid hormone action in the brain have allowed us to state, as modern neuroscientists, that *patterns* of gene expression govern *patterns* of behavioral functions.

To ultimately understand the differential role of these ER subtypes on complex behaviors, it will be critical to identify and explore the different molecules that function in ER transcription in the brain. Elucidating the myriad of modifications of chromatin elicited by these coactivators and other regulatory molecules in brain will be essential. Moreover, understanding how these proteins assemble at the promoter, in a brain region and cell-type specific fashion, will be an exciting challenge for neuroendocrinologists to tackle in the near future.

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