Androgen Receptors, Sex Behavior, and Aggression

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Key Words

Testosterone · Hypothalamic-pituitary-gonadal axis · Hypothalamic-pituitary-adrenal axis · Estrogen

Abstract

Androgens are intricately involved in reproductive and aggressive behaviors, but the role of the androgen receptor in mediating these behaviors is less defined. Further, activity of the hypothalamic-pituitary-gonadal axis and hypothalamic-pituitary-adrenal axis can influence each other at the level of the androgen receptor. Knowledge of the mechanisms for androgens' effects on behaviors through the androgen receptor will guide future studies in elucidating male reproductive and aggressive behavior repertoires.

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Androgen Receptors in Brain

Androgen receptors (ARs) are highly conserved and are present in organisms ranging from yeast to humans [1]. A vital role of ARs is the neuromodulation of sexual and possibly aggressive behaviors [2]. In this review we will discuss current knowledge of the effects of ARs on sexual and aggressive behaviors. Further, we will examine the role of the hypothalamic-pituitary-adrenal axis

(HPA) on hypothalamic-pituitary-gonadal (HPG) function at the level of the AR.

Androgen Mode of Action

Androgens are members of the steroid receptor superfamily of transcription factors [3]. Androgens, such as testosterone, act at receptor sites in specific brain areas [4]. The primary mode of action is regulation of gene transcription. Androgens enter the cell, bind to the cytosolic AR, and induce a conformational change that causes the dissociation of heat shock proteins and translocation of the receptor from the cytosol into the nucleus, and finally dimerization of the receptor [5]. The AR dimer binds to a specific DNA sequence, known as the hormone response element, resulting in up- or downregulation of gene transcription [4]. The AR is generally expressed as a single AR, and consists of a N-terminal regulatory domain, DNA-binding domain, small hinge region, and ligand-binding domain [6]. The N-terminal regulatory domain mediates most of AR's transcriptional activity [7].

Androgens can have a second mode of action, which is similar to estrogens having actions that are independent of genomic DNA interactions [8]. Based on rapid actions of testosterone, nongenomic effects of androgens were proposed over 30 years ago [9]. Further, ARs have been localized at extranuclear sites, such as hippocampal dendritic spines [10] and axons of the cerebral cortex [11].

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0028-3835/12/0962-0131\$38.00/0

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These extranuclear sites and rapid effects of testosterone indicate the presence of a putative membrane-associated AR (mAR) [12]. In a further indication that mAR may be different from AR, mAR is sensitive to G protein-coupled receptor antagonists but not AR antagonists [flutamide (FLU)] [13, 14]. Functionally, mAR has been associated with increased intracellular calcium [14]. Additionally, chemically modified exogenous androgens that are used clinically and illicitly as performance-enhancing compounds (anabolic androgenic steroids) have significant effects through allosteric modulation of neurotransmitter receptors, such as the GABA-A receptor [122]. Therefore, these data show that AR action may occur in a broader spectrum than previously believed.

Sites of Androgen Action

Distribution of AR mRNA containing cells has been identified in the forebrain, midbrain, brain stem, and spinal cord [15, 16]. Further, high concentrations of ARs were present in the medial preoptic area (MPOA), ventromedial hypothalamus (VMH), medial amygdala (AMY), nucleus accumbens, bed nucleus of stria terminalis (BNST), and septum (septum) as demonstrated by AR binding, immunohistochemistry, and in situ hybridization [16–24]. It is notable that the MPOA and the medial AMY have been implicated in the neural control of male sexual behavior [25], whereas the septum, bed nucleus of stria terminalis, and nucleus accumbens are associated with aggression regulation [26–28].

Androgen Receptor Blockade

The most popular method employed to investigate the effects of ARs on behavior is via antiandrogens. Although many antiandrogens are available, studies on behavior have focused on those that (1) have no detectable androgenic activity, (2) no antigonadotropic activity, and (3) can cross the blood-brain barrier (unless they are implanted intracranially). The most specific and widely used androgen receptor blockers have been FLU, FLU's active metabolite, hydroxyflutamide (OHF), and cyproterone acetate (CA) [29]. CA (1,2 α -methylene-6-chloro-17 α -acetoxyprogna-4,6-diene-3,20-dione) is a steroidal androgen receptor antagonist with weak progestational and glucocorticoid activity [30]. Both FLU and CA do not interfere with 5 α -dihydrotestosterone formation, but rather block androgen binding to the AR [31–33]. In contrast to CA, FLU [a,a,a-trifluoro-2-methyl-4'-nitro-m-propionotoluidide (or 4'-nitro-3'-trifluoromethylisobutyranilide)] is considered a pure antiandrogen, as it is devoid of antigonadotropic [34], progestogenic, estrogenic, and androgenic ac-

tivities [34, 35]; however, recent reports have shown that FLU and CA can exert some agonist activity at the level of the AR in neuronal and nonneuronal cells [36–39]. FLU's active metabolite, hydroxyflutamide (OHF; formerly SCH16423: a,a,a-trifluoro-2-methyl-4'-nitro-m-lactotoluidide) [35, 40], has been widely used as an antiandrogen as well. OHF has been shown to inhibit cell nuclear AR binding in pooled tissue from the hypothalamus, preoptic area, medial AMY, and septum [32, 41].

Sexual Behavior and Androgen Receptors

Virtually all reproductive-related behaviors, including copulation, aggression, scent marking, and ultrasonic vocalizations, are androgen-dependent [42]. Thus, they are facilitated in the presence of androgens (both endogenous and exogenous) and decline following castration [21, 32]. However, it appears that separate and perhaps overlapping brain regions mediate different androgen-dependent behaviors, and even different components of these behaviors.

Studies Using Systemic Antiandrogen Administration

Antiandrogens have been given systemically to determine if blocking ARs in the entire brain would suppress male sexual behavior. Timing of antiandrogen exposure, with respect to hormonal status, has been shown to be important. In some studies, for example, antiandrogens and testosterone replacement were instituted at the time of castration, while in other studies administration occurred 3 weeks following castration, in which male rats were in an androgen-deficient condition. In testosterone-treated castrated males, administration of both FLU and OHF at the time of castration was only marginally effective in suppressing male sexual behavior [32]. However, when antiandrogens and testosterone replacement were instituted 3 weeks following castration in androgen-deficient castrated male rats, male sexual behavior was inhibited [32, 41, 43].

The vast majority of studies have focused on the performance aspect or absence of male sexual behavior. However, two important factors to consider are the motivational component of male sexual behavior and reproductive-associated behaviors. In the absence of sexual motivation, copulation is greatly diminished [41]. Restoration of sexual motivation with androgens, as measured by partner preference, can be prevented by FLU [41]. With respect to reproductive-associated behaviors, systemic administration of OHF decreases ultrasonic vocal-

izations and scent marking [41], but the neural sites involved have yet to be identified. Thus, ARs appear to play a critical role in the expression of sexual behavior, sexual motivation, and reproductive-associated behaviors in rats.

Studies Using Antiandrogen Implants to Localize AR Effects

The benefit to using intracranial antiandrogen implants is that the entire brain is exposed to testosterone, whereas ARs are blocked only at the site of interest by the intracranial implant. This has proven to be a valuable method for identifying sites of AR action in the brain with regard to specific behaviors, as implanted animals can be tested for their behavioral response in the presence of the blocking agent.

The Medial Preoptic Area

The MPOA has long been associated with male sexual behavior and has high concentrations of ARs [20–24]. A role for AR activation in the MPOA in mediating male sexual behavior has been examined using intracranial implants. Testosterone implants into the MPOA reinstate male sexual behavior in castrates [44]. Intracranial implants of OHF into the MPOA results in a substantial reduction in mounts, intromissions, and ejaculations in a testosterone-treated castrated male rats [45–47]. Notably, implants of OHF into the MPOA decrease sexual motivation only when placed in the posterior aspect of this brain site [46]. Although far fewer studies have examined sexual motivation, it appears that both sexual performance and sexual motivation are under the control of ARs, though their site specificity may differ. It should be noted that AR is not the only steroid receptor in the MPOA that mediates male sex behaviors, as the estrogen receptor (ER) also mediates male sex behaviors, possibly relying on ER activation owing to estrogens synthesized locally by aromatase. Interestingly, it has been shown that testosterone can increase aromatase activity, while castration decreases aromatase activity in the MPOA [48]. Further, AR inhibition with FLU can decrease aromatase activity in the MPOA, thus indicating that aromatase activity, and by extension ER activation, may be AR-dependent in the MPOA [48].

Inhibition of testosterone conversion to estrogen by infusion of the aromatase inhibitor fadrozole into the MPOA of gonadally intact male rats decreased sex behaviors [49], and sex behaviors were restored with MPOA implants of estrogen and estrogen-BSA conjugate, suggesting that nongenomic mechanisms mediated estrogen

action [50]. Thus, both AR and ER in the MPOA are critical for the expression of male sexual behavior.

AMY and Olfactory Bulbs

Olfactory bulbs and the AMY are intimately connected and both contain ARs [51]. Removal of the olfactory bulbs drastically decreases male sexual behavior [51]. Moreover, olfactory bulb ablation significantly decreases AR binding in the AMY 1–2 days after surgery [51]. This decrease in AR binding was correlated with a decline in sexual behavior [51]. Implants of OHF into the medial AMY were moderately effective in suppressing male sexual behavior [45], suggesting that AR activation in the AMY may play a secondary role in facilitating male sexual behavior. Alternatively, the AMY may be important in some other, yet to be identified, component of male reproductive behavior.

Septum

In spite of the relatively high concentration of ARs in the septum [45, 46], blocking ARs in this brain area had no effect on either performance or motivational components of male sexual behavior. This suggests that other androgen-dependent behaviors may be mediated by the septum.

Ventromedial Hypothalamus

Few studies have examined the role of the VMH in male sexual behavior despite its high concentration of ARs. Testosterone implanted into the VMH did not restore copulation in castrated male rats [44]. This is interesting because OHF placed into the VMH is very effective in blocking male sexual behavior [45]. A later study showed that OHF in the anterodorsal VMH, but not the posteroventral VMH, decreased male sexual behavior [47]. Notably, VMH implants of testosterone were effective in restoring sexual motivation, and OHF in the VMH decreased sexual motivation [44, 45]. These results suggest a role for the VMH in modulating sexual motivation.

Testosterone and Estrogen

A number of years ago, a large body of evidence emerged suggesting that the male sex hormone was estrogen and not testosterone. This was referred to as the aromatization hypothesis, as testosterone must be metabolically converted to estradiol via the P450 aromatase enzyme in the brain to activate male sexual behaviors [52, 53]. The basis for this hypothesis was (1) very large doses of estrogen restored male sexual behavior [54] and (2) the aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD) blocked the effects

of androgens on male sexual behavior [55]. Later studies demonstrated that physiological doses of estradiol did not restore male sexual behavior, whereas physiological doses of testosterone were very effective [21]. Only testosterone restored ejaculation and increased AR occupation [21]. The nonaromatizable metabolite of testosterone, dihydrotestosterone (DHT), increased AR occupation, but had no effect on male sexual behavior [21]. In one study, the aromatase inhibitor fadrozole was given alone or in combination with testosterone, or with the addition of a very small dose of estradiol [56]. This experiment showed that blocking aromatization did indeed prevent the restoration of male sexual behavior, but not sexual motivation. The combination of testosterone with fadrozole and a very small dose of estradiol restored sexual behavior. However, estradiol alone had no effect on either sexual behavior or sexual motivation [56]. It was concluded that both testosterone and a small amount of estradiol through aromatase are needed to facilitate male sexual behavior.

Animals lacking in functional ARs have been used to study the role of androgen receptor activation in mediating male sexual behavior. The results have been somewhat equivocal, but this may be related to the fact that the defect is present from birth, rather than adulthood. Therefore, one cannot rule out the possibility that the changes in behavior are influenced by early development. In a study on male mice lacking a functional AR gene [57], both male sexual behavior and sexual motivation were low. Testicular feminized (Tfm) mice and rats, which are genetic males with defective ARs, have been used to study the effect of loss of AR function on male sexual behavior. Tfm mice lack functional ARs due to a mutation of the AR gene that results in a shortened AR transcript and no AR protein [58, 59]. Although these mice lack sensitivity to androgens [60], they still have circulating androgens, such as testosterone [61]. Further, Tfm mice have a greater AR defect than Tfm rats and also exhibit less male sexual behavior than Tfm rats [1]. Tfm rats show reduced sexual behavior, but not motivation [62]. Both Tfm mice and rats have normal levels of ER binding [1], as well as decreased aromatase activity that is unaffected by testosterone administration [63]. These results suggest that Tfm males have normal ER capacity, but not ER activation due to decreased aromatase activity. This is significant because estrogens have been shown to play a role in male sexual behavior. Consistent with these studies, male sex behaviors were decreased and ER α expression was unaffected in a conditional AR inactivation mouse model that is unaffected by development [64]. The foregoing data are fairly convincing in demonstrating a

role for ARs and possibly ER in mediating male reproductive responses. The mechanism by which estrogens contribute to AR action is unknown.

Aggression and Androgen Receptors

Male aggression is mediated by androgens, and elimination of endogenous androgens by castration abolishes aggression in a variety of species [2]. In spite of the extensive literature demonstrating the critical relationship between androgens and aggression, very little is known about the role of the AR as well as the specific neural sites of AR activity that are either necessary or sufficient to elicit aggression in males. The application of antiandrogens or AR antagonists, CA and FLU, has been used to assess this relationship. The effects of CA on aggression have been studied using animal models. However, the results have been equivocal. For example, the antiandrogen CA reportedly decreased intermale aggression in mice [65] and gerbils [66], whereas in other studies there was no significant effect on aggression [67–69]. Others have reported that male mice given CA did show an initial increase in fight latency, but decreased intermale aggression was evident only after 4 weeks [70]. These authors suggest that extensive exposure to the antiandrogenic blocking agent is necessary to suppress aggression [70]. This contrasts with one other study in which long-term CA treatment was administered to mice exposed to increasing doses of testosterone where no effect on aggression was found [71]. However, based on this finding it is not clear whether the sustained aggression was due to the inability of CA to suppress aggression or whether the increasing testosterone exposure was sufficient to override the inhibitory effect of CA.

The AR antagonist FLU also failed to decrease aggression in castrated male mice given exogenous testosterone [33]. Further, FLU failed to reduce the level of aggression in male castrated mice given the anabolic steroid methyltestosterone [72]. However, FLU did have an inhibitory effect on the ventral prostate, seminal vesicles, and lavalator ani weights compared to males given methyltestosterone alone, suggesting FLU differentially affects androgen-sensitive tissue and behavior [72]. The assessment of AR in mediating male aggression was also examined using mice lacking functional androgen receptors (Tfm). Robinson et al. [73] found that Tfm male mice did not display enhanced aggression following administration of various anabolic steroids, indicating that the presence of a functional AR may be necessary for androgens to elicit

aggressiveness in males. However, the ER has also been reported to mediate aggression in males. Exogenous estrogen administration in castrated Tfm mice and wild-type mice increased aggression [74]. Further, ER α activation is associated with increased aggression, while ER β activation is associated with decreased aggression [74, 75]. While these findings implicate both AR and ER in the evocation of aggressive behaviors, both the relative contribution of AR activation and the specific neural sites involved are poorly understood.

HPA System Involvement

Stress can inhibit sex steroid hormone-dependent physiology and behavior [76–78]. The neuroendocrine system involved in mediating responses to stress is the HPA, which consists of direct influences and feedback interactions between the hypothalamus, pituitary, and adrenal gland [79]. Upon activation of the HPA axis, the paraventricular nucleus of the hypothalamus and the pituitary secrete corticotropin-releasing hormone and adrenocorticotrophic hormone, respectively [79]. These hormones act upon the adrenal gland to produce glucocorticoid hormones, such as corticosterone [79]. Through a negative feedback cycle, glucocorticoids can act on receptors in the hypothalamus and pituitary to suppress adrenocorticotrophic hormone and corticotropin-releasing hormone secretion [79]. Glucocorticoids can act at two receptors in the brain: mineralocorticoid receptors and the glucocorticoid receptors [80]. These receptors are widely distributed in many brain regions that contain high concentrations of ARs, such as the hypothalamus, hippocampus, cortex, AMY, and septum [15, 16, 80], indicating the involvement of glucocorticoids in other physiological functions.

Sex Steroid Hormone-Dependent Behaviors and Stress

It is known that HPA activation can inhibit HPG activation, i.e. reproductive physiology and associated behaviors [76–78, 81]. However, HPG activation can also inhibit HPA activation. Brain regions, such as the hypothalamus and AMY, which are activated by stress and express glucocorticoid receptors, can also be activated by sexual experience [80, 82–85]. Studies have shown that prior HPG activation, through aggression or sexual experience can decrease stress reactions [86, 87]. Interestingly, even exogenous manipulation of the HPG axis, via administration of testosterone, can block stress-induced glucocorticoid increase [88]. These studies indicate that the HPA and the HPG systems interact and influence each other.

Androgen Receptors and Stress

This interaction between the HPA and HPG systems could be mediated by AR activation (fig. 1). AR has been shown to be involved in anxiety-related behaviors in rodents [89, 90]. Studies have shown that AR activation inhibits stress response, while AR inhibition can increase stress response [89, 90]. Consistent with behavioral studies, mice lacking androgen receptors (ARKO mice) have increased HPA activation, as evidenced by increased corticosterone and adrenocorticotrophic hormone release [91]. Even modulation of the HPG system, via AR inhibition, during the perinatal critical period can alter HPA function in adulthood [92, 93], indicating that HPG-associated AR activation during the perinatal period is essential for adult HPA function [94].

Testicular Feminization Mutation

Tfm (testicular feminization mutant) mice are a useful model to study HPG and HPA interactions. Consistent with previous reports that found increased HPA activation in response to pharmacological AR inhibition [89, 90], Tfm mice also display increased HPA activation [95, 96], such as increased anxiety-associated behaviors and corticosterone levels [95]. Interestingly, the locus coeruleus, a brain region implicated in the stress response, contains more neurons and has a larger volume in a Tfm rat model compared to wild-type [97], indicating that the lack of HPG-associated AR activation results in an overactivation of the HPA system. Results from studies using either pharmacological or genetic inhibition of the AR have consistently shown that an AR-mediated mechanism underlies HPA and HPG interactions.

Looking Forward

In this section of the review we will discuss two unresolved issues related to AR action and sociosexual behaviors: (1) a potential role for the ER in activating male sexual behavior, and (2) the reason for the long lag time for the cessation and restoration of male sexual behavior.

Effects of ER Inhibition

The permissive role of estrogen in facilitating the display of male sexual behavior is poorly understood. The implication is that estrogen acts via the ER. Further, studies have shown that estrogens, possibly through the ER, can upregulate AR expression both centrally (hypothalamus and AMY) and peripherally (prostate) [98, 99]. The most powerful evidence for a role of ER in mediating

Fig. 1. AR control of HPG and HPA axis. Activation of the HPG axis results in increased AR activation, resulting in decreased activation of the HPA axis. Conversely, HPA axis activation decreased AR activation and subsequent HPG activation. HPA and HPG axis can modulate each other at the level of the AR. GnRH = Gonadotropin-releasing hormone; LH = luteinizing hormone; FSH = follicle-stimulating hormone; ACTH = adrenocorticotrophic hormone; CRH = corticotropin-releasing hormone.

male sexual behavior comes from ER knockout mice [100–102]. In ER α knockout mice, male sexual behavior and aggression were dramatically reduced [100, 101]. However, sexual motivation remained normal [100–102]. Interestingly, ER β knockout mice display normal male sexual behaviors, thus suggesting an ER α - rather than ER β -mediated mechanism [103]. The problem is that it is impossible to determine if the ER-mediated effects on sexual and aggressive behaviors is due to organizational or activational effects of estrogen acting at the ER, as ER and estrogens are known to play an integral role in the organization of the brain with regard to adult behavioral patterns [1].

The use of ER antagonists has proven to be beneficial to parse out the role of estrogen. Commonly used ER antagonists include: ICI 182,780, CI-628, RU-58668, PHTPP, and MPP. RU-58668 and ICI 182,780 are both pure ER α and ER β antagonists with no partial agonist activity [104, 105]. RU-58668 is able to cross the blood-brain barrier [106], unlike ICI 182,770 [107]. The ER antagonist CI-628 can inhibit ER α and ER β , but has partial agonist activity [108]. Lastly, MPP (methyl-piperidino-pyrazole) is a spe-

cific ER α antagonist [109], whereas PHTPP is a selective ER β antagonist [110]. In Japanese quails, the ER antagonist CI-628 decreased male sex behaviors and aggression [111–113], but the results are equivocal in rodents with regard to CI-628 [114–116] and RU-58668 [41]. No studies have examined ER antagonism on specific brain nuclei or the role of ER α and ER β on male sex behaviors and aggression. Therefore, the question of a role for the ER in facilitating the expression of male sexual behavior remains unanswered.

Proteins

One of the most intriguing but elusive aspects of male sexual behavior is the reason for the long lag time for the decline of sexual behavior following castration, and the restoration of sexual behavior after hormone replacement therapy. In rats, testosterone levels fall to undetectable levels in less than 24 h [117]. Thus, the gradual decline of sexual behavior over 2–3 weeks is not due to the presence of testosterone. Following the reinstatement of daily testosterone therapy, the restoration of the full copulatory behavior pattern requires 7–14 days [32, 55, 118].

This is a long time considering that female receptivity can be reinstated within 48 h [119]. With regard to male sexual behavior, a 'critical exposure' time of approximately 21 h per day for 7–10 days is needed to restore ejaculation [118]. Neural ARs were significantly decreased within 3 h of testosterone-capsule removal and was at castration levels by 6 h. This suggests that high levels of AR must be maintained over time to activate male sexual behavior.

It is known that in many species, including humans, prior sexual experience plays a crucial role in maintaining sexual behavior following castration. The more experience, the longer sexual behavior is maintained [120]. One possibility is that proteins induced by androgens are essential for the maintenance of the behavioral response. While this is a plausible mechanism, it has proven extremely difficult to demonstrate the presence of androgen-regulated proteins in brain. In our laboratory, we used various hybridization and PCR techniques to identify four proteins that were upregulated and two proteins that were downregulated by testosterone in the hypothalamus (unpubl. observations). However, we were unable to conclusively demonstrate that these were androgen-regulated gene products. Thus far, no one has been able to definitively link brain proteins to male sex behavior. This problem has plagued researchers for decades and is still unresolved.

Conclusion

Localization and pharmacological inhibition of ARs have provided overwhelming evidence of AR involvement in male reproductive function and associated behaviors, as well as knowledge of a feedback system involving both the HPA and HPG circuits. However, there is much to be learned and many unknowns of AR action, such as (1) which specific neural sites mediate what specific aspects of reproductive behavior, (2) what is the mechanism of action for estrogen's effects on AR activation, (3) the role of the AR in aggressive behaviors, and (4) the impact on aging on AR action, since testosterone levels decrease with age [121]. The strength of the previous findings on the effects of ARs on reproductive and aggressive behaviors would greatly benefit by addressing these questions.

Acknowledgements

This work was supported by the following grants: NIH grants 27727 to M.Y.M.; and NIH F32 NS061417, AHA BGIA4180116, and UNTHSC Seed Grant to R.L.C. The authors would like to thank Ms. Kathryn M. Cunningham for her artwork.

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