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Testosterone regulation of Alzheimer-like neuropathology in male 3xTg-AD mice involves both estrogen and androgen pathways

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Abstract

Normal, age-related depletion of the androgen testosterone is a risk factor for Alzheimer's disease (AD) in men. Previously, we reported that experimental androgen depletion significantly accelerates development of AD-like neuropathology in the 3xTg-AD triple-transgenic mouse model of AD, an effect prevented by androgen treatment. Because testosterone is metabolized in brain into both the androgen dihydrotestosterone (DHT) and the estrogen 17β-estradiol (E2), testosterone can mediate its effects through androgen and or estrogen pathways. To define the role of androgen and estrogen pathways in regulation of AD-like neuropathology, we compared the effects of testosterone (T) and its metabolites DHT and E2 in male 3xTg-AD mice depleted of endogenous sex steroid hormones by gonadectomy (GDX). Male 3xTg-AD mice were sham GDX or GDX, immediately treated with vehicle, T, DHT, or E2, and 4 months later evaluated for two indices of AD-like neuropathology, β-amyloid (Aβ) accumulation and tau hyperphosphorylation. In comparison to sham GDX mice, we observed a significant increase in Aβ accumulation in GDX mice in subiculum, hippocampus, and amygdala. Treatment of GDX mice with T prevented the increased Aβ accumulation in all three brain regions. DHT treatment yielded similar results, significantly reducing Aβ accumulation across brain regions. Interestingly, E2 prevented Aβ accumulation in hippocampus but exerted only partial effects in subiculum and amygdala. Levels of tau hyperphosphorylation in sham GDX male 3xTg-AD mice were modest and only slightly increased by GDX. Treatment of GDX mice with T or E2 but not DHT reduced tau hyperphosphorylation to levels lower than observed in sham animals. These data suggest that testosterone regulates Aβ pathology through androgen and estrogen pathways and reduces tau pathology largely through estrogen pathways. These findings further define hormone pathways involved in regulation of AD-related pathology, information that is important for understanding disease etiology and developing pathway-specific hormone interventions.

Keywords

Alzheimer's disease; β-amyloid; sex steroid hormones; tau hyperphosphorylation; transgenic mouse

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1. INTRODUCTION

Advancing age is the most significant risk factor for the development of Alzheimer's disease (AD) (Evans et al., 1989; Jorm et al., 1987; Rocca et al., 1986). One age-related change in men that has been identified as a risk factor for the development of AD is significant depletion in the primary male androgen, testosterone (T) (Pike et al., 2009; Rosario and Pike, 2008). The association between low testosterone and increased risk for AD likely reflects the loss of beneficial androgen-mediated actions in the brain. There are several neural androgen actions potentially relevant to AD, including promotion of neuron viability (Pike, 2001; Ramsden et al., 2003b), synaptic plasticity (Leranth et al., 2003), select aspects of cognition (Cherrier et al., 2005; Janowsky, 2006b; Moffat, 2005), and reduction of tau phosphorylation (Papasozomenos, 1997). Of particular importance is androgen regulation of Aβ, an hypothesized casual factor in the development of AD (Hardy, 1997). Elevated levels of Aβ have been observed in men with low levels of testosterone (Almeida and Papadopoulos, 2003; Gandy et al., 2001; Gillett et al., 2003; Rosario et al., 2009), suggesting that testosterone may function to decrease Aβ levels. Consistent with this possibility, cell culture studies have shown that T treatment reduces Aβ levels (Gouras et al., 2000; Yao et al., 2008). Importantly, findings in animal models also demonstrate that androgens are negative regulators of Aβ. Depletion of endogenous androgen levels by gonadectomy (GDX) resulted in a significant increase in soluble Aβ in wild-type male rats (Ramsden et al., 2003a) and elevated Aβ accumulation in male 3xTg-AD mice (Rosario et al., 2006), effects that were prevented by androgen treatment. Thus, regulation of Aβ is a neural androgen function that is compromised as a consequence of age-related T depletion and may contribute to AD pathogenesis.

An important issue that remains to be resolved is the role of androgen versus estrogen pathways in androgen regulation of AD pathology. Testosterone not only directly interacts with the androgen receptor (AR), but also acts as a prohormone, undergoing metabolism in brain to a variety of hormones that act by several mechanisms including activation of AR and estrogen receptors (ERs) (Attardi et al., 1976; Souttou et al., 1993). In particular, T is converted by aromatase to the estrogen 17β-estradiol (E2) and by 5α-reductase to the potent androgen dihydrotestosterone (DHT), hormones that specifically activate ERs and AR, respectively. Available cell culture evidence suggests that both estrogen and androgen pathways may be involved in androgen regulation of Aβ. First, testosterone has been shown to reduce Aβ levels in cultured neural cells by altering amyloid precursor protein processing (Gouras et al., 2000), however this action was inhibited by an aromatase inhibitor that prevents conversion of T to E2 (Goodenough et al., 2000). Second, we recently demonstrated that androgens reduce Aβ levels in culture by an AR-dependent mechanism involving increased expression of the Aβ-degrading enzyme neprilysin (Yao et al., 2008). To evaluate the potential involvement of androgen and estrogen pathways in regulation of AD-like neuropathology, we compared the effects of T, DHT, and E2 on levels of Aβ accumulation and tau hyperphosphorylation in male 3xTg-AD mice. Hormone levels were manipulated between ages 3–7 months, a time frame which corresponds to the early phases of pathology in male 3xTg-AD mice (Oddo et al., 2003a; Rosario et al., 2006), and thus allows for evaluation of androgens and estrogens on the development of AD-like pathology.

2. RESULTS

2.1 Efficacy of hormone manipulations

To evaluate the contribution of androgen and estrogen pathways in the regulation of AD-like pathology, young adult male 3xTg-AD mice were GDX to deplete endogenous sex steroid hormones, and then treated with T, DHT or E2 at previously established doses known to increase androgen or estrogen activity (Carroll et al., 2007; Rosario et al., 2006). In order to

confirm the efficacy and specificity of the hormone manipulations, we measured seminal vesicle weight, a sensitive bioassay of androgen levels (Yamane et al., 1986). We observed a significant decrease in seminal vesicle weight in GDX mice relative to sham GDX mice (sham GDX, 63.5 ± 9.2 mg; GDX, 13.3 ± 1.4 mg, F (2,27) = 6.8, p <0.05). In comparison, the GDX +DHT group showed seminal vesicle weights (128.2 ± 20.1 mg) significantly higher than both the GDX and sham GDX groups (p <0.05), indicating that DHT treatment resulted in high physiological or supraphysiological DHT levels. Because the T pellets were depleted before their designed 60 day period (data not shown), we assessed T pellet efficacy in GDX mice 30 days after pellet implantation and found seminal vesicle weights significantly higher than GDX mice but not sham GDX mice, suggesting physiological replacement of T (GDX+T, 49.5 ± 7.6 mg F (3,15) = 9.1, p <0.05). E2 had no significant effect on seminal vesicle weight as compared to GDX or Sham GDX mice (GDX+E2, 24.2 ± 3.4 mg, p <0.05).

2.2 Hormone regulation of Aβ deposition

To determine whether androgen regulation of Aβ pathology is mediated by androgen and or estrogen pathways, we examined the effects of the hormone manipulations on Aβ deposition in three brain regions known to be affected by Aβ pathology in 3xTg-AD mice and human AD, the subiculum, hippocampus CA1, and amygdala. Statistical analysis with repeated measures ANOVA indicated a significant effect of condition on Aβ load across brain regions [F = 2.95 (8,62), p < 0.01]. Specifically, we observed a significant increase in the intensity and area of Aβ immunoreactivity in androgen depleted GDX mice in comparison to sham GDX animals in the subiculum (Fig. 1), CA1 region of hippocampus (Fig. 2), and amygdala (Fig. 3). Treatment of GDX mice with DHT prevented the increased Aβ accumulation in all three brain regions that is observed in GDX mice, yielding Aβ load values at or below the levels observed in the sham GDX group (Figs. 1–3). Testosterone also significantly prevented Aβ accumulation in comparison to GDX mice with the same regional efficacy and a similar magnitude as observed in the GDX+DHT group. The GDX +E2 group showed mixed effects when compared with GDX mice. In hippocampus CA1, E2 treatment significantly attenuated Aβ accumulation with an efficacy similar to that of T and DHT (Fig. 2). However, in both subiculum and amygdala, E2 treatment resulted in Aβ load values that were not significantly different from either the sham GDX or GDX groups (Figs. 1, 3), suggesting partial efficacy.

In addition to Aβ load, we also counted numbers of extracellular plaques as a measure of Aβ deposition. Although we observed very few plaques in male 3xTg-AD mice at age 7 mo, there was a significant hormone-related effect on extracellular plaque number [F *(5,45)* = 3.32, p <0.05]. Androgen depletion significantly increased the number of extracellular plaques (sham GDX, 0.09 ± 0.091; GDX, 1.0 ± 0.35), an effect prevented by both T and DHT treatment (GDX +T, 0.14 ± 0.14; GDX+DHT, 0.0 ± 0.0). E2 had no effect on the number of extracellular plaques in GDX mice (GDX+ E2, 1.0 ± 0.45).

2.3 Hormone regulation of tau hyperphosphorylation

To evaluate tau hyperphosphorylation across the previously described treatment groups in the male 3xTg-AD mice, we counted numbers of cells strongly immunoreactive for the antibody AT8, which recognizes phosphorylation of tau at Ser^{202} and Thr^{205}. Consistent with previous reports (Oddo et al., 2003a; Oddo et al., 2003b), we observed a significant age-related increase in AT8-immunreactive neurons, which were predominantly localized in subiculum and to lesser extent hippocampus CA1 (Fig. 4A–E). At age 7 mo, we observed modest numbers of AT8-labeled cells which showed a statistically non-significant increase in the GDX group. Interestingly, the GDX+T and GDX+E2 groups exhibited significantly

fewer numbers of AT8-immunoreactive neurons than both the sham GDX and GDX groups (Fig. 4F). DHT had no significant effect on the number of AT8-immunoreactive neurons.

3. DISCUSSION

In this study, we examined whether the recently established androgen regulation of AD neuropathology is mediated through androgen and or estrogen pathways. Our findings indicate sex steroid regulation of Aβ, evidenced by a significant increase in intraneuronal and extracellular Aβ pathology following GDX-induced androgen depletion. Further, the data suggest that hormonal regulation of Aβ is mediated largely via activation of androgen pathways although E2 also exhibited Aβ-lowering actions. In contrast, regulation of tau hyperphosphorylation appeared to be regulated predominantly by estrogen pathways as numbers of pre-tangle neurons were reduced by T and E2 but not DHT. These data indicate that T utilizes both androgen and estrogen pathways to reduce indices of AD pathology in male 3xTg-AD mice.

3.1 Roles of androgen and estrogen pathways in androgen regulation of Aβ

Consistent with our previous work (Rosario et al., 2006), we found that androgen depletion accelerates the development of Aβ pathology in the 3xTg-AD mouse model of AD, findings which implicate androgens as endogenous regulators of Aβ. Our observation that T prevented the GDX-induced increase in Aβ pathology confirms androgen regulation of Aβ but does not define the relative involvement of androgen and estrogen pathways. This is because T functions in brain as a prohormone, converted by aromatase into the estrogen 17β-estradiol and by 5∞-reductase into the potent androgen DHT. Like T, DHT is also a substrate in brain for enzymes whose actions generate active hormones. Of particular interest, DHT can be converted to 5α-androstan-3β,17β-diol, which is an agonist for estrogen receptor β (ERβ) (Weihua et al., 2001; Weihua et al., 2002) and mediates some neural effects of DHT (Handa et al., 2008; Handa et al., 2009; Lund et al., 2006; Pak et al., 2005). Thus, both the T metabolite 17β-estradiol and the DHT metabolite 5α-androstan-3β, 17β-diol are ER agonists that activate estrogen pathways and may contribute to observed Aβ-lowering effects of T and DHT.

Supporting a role of androgen pathways in Aβ regulation, we observed that T and DHT treatments similarly impacted Aβ accumulation in a manner that significantly differed from E2 treatment. Further comparisons on the relative efficacies of T and DHT are not possible from our study since these androgens differ in their affinities for AR and the absolute levels of the hormones were not assessed. It is also important to note that the utilized method of hormone delivery has limitations. Subcutaneous pellets are advantageous in that they allow continuous hormone treatment for extended periods without repeated invasive procedures, however they can also yield uneven hormone delivery with very high initial levels and low final levels (Strom et al., 2008, 2009).

Whereas both T and DHT strongly inhibited the elevated Aβ accumulation induced by GDX across all three examined brain regions, E2 treatment was associated with more modest Aβ reduction that was statistically significant only in hippocampus CA1. Thus, we conclude that androgens can lower Aβ by mechanism(s) independent of estrogen pathways. Although the experimental design does not exclude the possibility that DHT may indirectly activate estrogen pathways via metabolism to 5α-androstan-3β,17β-diol which acts on ERβ, we have previously reported that Aβ levels in hippocampus CA1 and subiculum in female 3xTg-AD mice were reduced by an ER∞ agonist but not an ERβ agonist indicating ER∞ is more important in Aβ regulation (Carroll and Pike, 2008). Further, we previously found that GDX-induced elevation of soluble Aβ in male Sprague-Dawley rats was prevented by DHT but not E2 treatment (Ramsden et al., 2003a). Also consistent with an androgen pathway of

Aβ regulation is recent work in guinea pigs that demonstrates an inverse relationship between levels of soluble Aβ in cerebrospinal fluid and serum levels of T (Wahjoepramono et al., 2008). Similarly, brain levels of Aβ in aged men are inversely associated with brain levels of T but not E2 (Rosario et al., 2009).

While the data suggest that androgens utilize an estrogen-independent mechanism to reduce Aβ levels, they also indicate involvement of estrogen pathway(s). Specifically, in GDX male 3xTg-AD mice, we found that E2 treatment resulted in significantly lower Aβ levels in hippocampus CA1 and intermediate Aβ levels in subiculum and amygdala that were between sham GDX and GDX groups. Since E2 is not an effective agonist for AR, these findings implicate an AR-independent estrogen pathway in regulation of Aβ levels in male brain. Interestingly, we observe that the same dose of E2 delivered to ovariectomized female 3xTg-AD mice significantly reduces Aβ accumulation not only in hippocampus CA1 but also in subiculum, amygdala, and frontal cortex (Carroll et al., 2007; Carroll and Pike, 2008). Unclear is why E2 was only partially effective in reducing Aβ in males. Although activation of estrogen pathways has several important functions in the male brain including regulation of aspects of development and sexual differentiation (Cooke et al., 1998; Forger, 2006), some established E2 actions in female brain are not observed in male brain. For example, E2 is an established regulator of spine density in female hippocampus (Woolley and McEwen, 1992; Woolley and McEwen, 1994) whereas DHT but not E2 increases spine density in male hippocampus (Leranth et al., 2003; Leranth et al., 2004). Thus, our data suggest that estrogen pathways contribute to androgen regulation of Aβ but that such pathways may be less effective in males than in females.

Although these data define contributions of both androgen and estrogen pathways in regulation of Aβ, they do not elucidate the underlying signaling mechanisms. Sex steroid hormones function largely via binding to and activating their specific steroid receptors, AR for T and DHT and ERα and ERβ for E2. As with other steroid hormone receptors, activation of AR and ER can initiate both classic genomic and rapid cell signaling pathways (Falkenstein et al., 2000). AR and ERs are localized in many brain regions, including hippocampus and amygdala (Kerr et al., 1995; Simerly et al., 1990), which were examined in this study. Although both ERα and ERβ are present in the hippocampus and amygdala, ERβ has been shown to be expressed at greater levels than ERα in both the hippocampus and amygdala (Mehra et al., 2005; Shughrue et al., 1997). Previous reports indicate that both AR and ER are involved in regulation of Aβ. In the case of AR, we observed that T and DHT reduced soluble Aβ in a cell culture paradigm by a AR-dependent genomic mechanism involving increased expression of the Aβ-catabolizing enzyme neprilysin (Yao et al., 2008). Similarly, *in vitro* and *in vivo* evidence has identified activation of ERβ as important to E2 regulation of Aβ (Carroll and Pike, 2008) by a mechanism involving increased expression of insulin degrading enzyme (Zhao et al., 2010), an Aβ-catabolizing protein. Besides these established AR- and ER-dependent pathways, the observed effects of T, DHT and E2 may involve other non-receptor dependent pathways. For example, sex steroid hormone regulation of Aβ could involve lowering levels of luteinizing hormone (LH) via regulation of the hypothalamic-pituitary-gonadal axis. Elevated LH is associated with AD (Bowen et al., 2000) and may have a role in modulating Aβ levels (Meethal et al., 2005). In addition, some neural actions of T and DHT are mediated by an AR-independent pathway involving DHT metabolism to 5α-androstan-3β,17β-diol which acts on GABAA receptors (Edinger and Frye, 2004). One limitation of this study is that it does not specifically define the relative roles of AR and ER as mediators of observed androgen and estrogen pathways.

3.2 Roles of androgen and estrogen pathways in androgen regulation of tau hyperphosphorylation

In contrast to several studies describing a relationship between androgens and inhibition of Aβ accumulation (Pike et al., 2009), few studies have investigated the possibility that androgens may reduce AD-like tau pathology. Notably, work by Papasozomenos and colleagues found that androgens can reduce tau hyperphosphorylation induced by acute heat shock in male rats (Papasozomenos, 1997; Papasozomenos and Shanavas, 2002). In this study, we observed a statistically nonsignificant trend of elevated tau phosphorylation in GDX males that was not apparent in the DHT group. Interestingly, in middle-aged male 3xTg-AD mice that exhibit more advanced tau pathology, we do observe a significant relationship between androgen depletion and increased tau pathology that is prevented by DHT (unpublished observations). Perhaps unexpectedly, we found that the modest numbers of AT8-immunoreactive neurons observed in the relatively young sham GDX mice from this study were significantly lower in groups receiving T but not in those receiving DHT. Further, E2 treatment was also associated with a significant decrease in tau hyperphosphorylation. Together, these data suggest a potential role for estrogen pathways in regulation of tau phosphorylation since both E2 and T (via aromatization to E2) treatments are expected to increase brain levels of estrogens. A role of estrogen pathways in reducing tau phosphorylation is consistent with our observations in female 3xTg-AD mice (Carroll et al., 2007). Further, E2 has been found to decrease tau phosphorylation in cell culture (Liu et al., 2008; Zhang et al., 2008), an effect inhibited by ER antagonism (Alvarez-de-la-Rosa et al., 2005). Interestingly, in the heat shock model used by Papasozomenos and colleagues (1997), testosterone inhibition of tau hyperphosphorylation was estrogen independent.

An important consideration in the interpretation of tau hyperphosphorylation data in the 3xTg-AD mouse is the relationship between tau and Aβ pathologies. Previous work in this AD mouse model has found that reduction of Aβ by Aβ immunotherapy also results in decreased levels of tau phosphorylation, suggesting Aβ accumulation contributes to tau pathology in the 3xTg-AD model (Oddo et al., 2003a; Oddo et al., 2006). However, tau hyperphosphorylation in 3xTg-AD mice can be reduced in the absence of changes in Aβ levels by directly targeting phosphorylation pathways (Caccamo et al., 2007; Kitazawa et al., 2005). Our observations are more consistent with the latter finding in which Aβ and tau are not inextricably linked. First, although DHT reduced Aβ levels this androgen treatment did not significantly affect tau pathology. Second, E2 only modestly reduced Aβ yet strongly lowered numbers of neurons with hyperphosphorylated tau. Thus, our data are consistent with the hypothesis that estrogen pathways can reduce tau pathology by affecting phosphorylation pathways, as suggested by in vitro studies (Alvarez-de-la-Rosa et al., 2005; Liu et al., 2008; Zhang et al., 2008).

3.3 Clinical implications: androgen therapy and AD

Age-related T depletion in men is a recently identified risk factor for the development of AD (Hogervorst and Bandelow, 2004; Moffat, 2005; Pike et al., 2009; Rosario et al., 2004). Testosterone-based androgen therapy is increasingly utilized in aging men to treat clinical manifestations of hypogonadism, including sexual function, muscle mass and strength, bone mineral density, cognition, and overall state of well being or quality of life (Bhasin et al., 2006; Bhasin et al., 2007; Kaufman and Vermeulen, 2005). Androgens also promote cognitive function in both men and male rodents (Janowsky, 2006a; Janowsky, 2006b), actions that apparently involve both androgen and estrogen pathways as evidenced by similarities and differences in the cognitive and behavioral effects of T and DHT (Benice and Raber, 2009; Bimonte-Nelson et al., 2003; Cherrier et al., 2003; Edinger and Frye, 2004; Frye et al., 2004). Given the present findings as well as the recent literature suggesting neuroprotective effects of androgens against AD-related pathology (Pike et al., 2009; Raber,

2008; Rosario and Pike, 2008), further clinical evaluation of androgen therapy to reduce the risk of AD and improve neural health in aging men with low T appears warranted. Because our data demonstrate that androgens utilize both androgen and estrogen pathways to reduce Aβ and tau pathologies, the most effective androgen therapies for combating AD-related pathologies may be T and selective androgen receptor modulators that are capable of acting through both androgen and estrogen pathways.

4. EXPERIMENTAL PROCEDURES

4.1 Animals and hormone treatments

We utilized adult male 3xTg-AD mice, a triple transgenic model that expresses disease-related mutations in amyloid precursor protein, presenilin 1, and tau (Oddo et al., 2003b), to examine the effect of T, DHT, and E2 on regulation of AD pathology. All the mice used in this study were bred in our laboratory, had *ad libitum* access to food and water, and were housed individually under a 12h light/12h dark schedule. Mice were divided into 5 treatment groups (N = 6–7 per group): sham GDX (treated with placebo), GDX (treated with placebo), GDX+T, GDX +DHT, and GDX+E2 and were maintained under these hormone conditions for 4 months, from 3 months of age until 7 months of age. Under pentobarbital (50 mg/kg) anesthesia, 3 mo-old male 3xTg-AD mice were either sham GDX or GDX and immediately implanted with slow-release, subcutaneous delivery pellets (Innovative Research of America, Sarasota, FL) containing placebo (hormone-free, 90-day pellet), DHT (10 mg, 90-day pellet), T (10 mg 60-day pellet) or E2 (0.025mg 60-day pellet). To maintain treatments over a 4-month period, animals were delivered a second pellet 2 mo (T, E2) or 3 mo (Pl, DHT) after the initial pellet. Both 60- and 90-day placebo pellets were utilized in Sham GDX and GDX groups; no apparent effects on pathology were noted between 60- and 90-day placebo pellets (data not shown).

Two additional groups of sham GDX animals at ages 3 mo and 13 mo were also included for tau analyses to identify any age-related changes in tau. At the end of the treatment period, mice were anesthetized with pentobarbital (100 mg/kg), blood was collected for hormone analyses, and animals were perfused with PBS. Seminal vesicles were dissected, blotted, and weighed to determine effectiveness of androgen treatments (Yamane et al., 1986). The brain was dissected and immersion fixed in 4% paraformaldehyde for immunohistochemical analyses. Animal studies were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and under an institutionally approved animal protocol.

4.2 Immunohistochemistry

Paraformaldehyde-fixed hemi-brains were sectioned in the horizontal plane (40 μm) using a vibratome. Tissue sections were immunostained in a few large batches in which sections from the different treatment groups were evenly distributed to control for inter-batch variability. In each experiment, sections were immunolabeled following a previously described protocol (Pike, 1999). Briefly, sections were quenched of endogenous peroxidase by H_2O_2 rinse then blocked for non-specific binding by incubation in TBS-Triton-X buffer containing 2% serum. For Aβ immunolabeling, sections were also pretreated with an antigen unmasking step (5 min rinse in 99% formic acid) to increase Aβ staining (Cummings, 2002). Sections were incubated overnight with buffer containing a primary antibody specific for either hyperphosphorylated tau (AT8 monoclonal antibody, 1:1000; Pierce, Rockford, IL) or Aβ (rabbit anti-human Aβ1-43, 1:300 dilution; Zymed, San Fransisco, CA). Immunoreactivity was visualized using the Vector Elite ABC kit followed by diaminobenzidine reaction according to manufacturer's instructions (Vector, Burlingame,

CA). Immunolabeled sections were dehydrated by sequential rinses in solutions with increasing alcohol content and cover-slipped in permanent mounting medium.

4.3 Quantification of immunohistochemistry

Immunohistochemistry was quantified by two methods. First, Aβ levels in immunostained sections were determined using an immunoreactive load technique previously described (Carroll et al., 2007; Rosario et al., 2006). In brief, high magnification fields (420 μm × 330 μm) from Aβ-immunolabeled sections were collected and stored on computer by a video capture system (B/W CCD camera coupled to an Olympus BX40 upright microscope). For each brain, images were collected for each brain region (subiculum, hippocampus CA1, amygdala) from 5 separate sections. In a regular pattern, 2 non-overlapping fields for subiculum and amygdala and 3 non-overlapping fields for CA1 were captured per section. Using NIH Image 1.61 software, gray scale images were processed with a constant threshold value to create a binary separation between positive and negative immunoreactivity such that each pixel was either black or white. This conversion permits calculation of the percentage of section area occupied by immunoreactive label (i.e., load); it is not a measure of immunoreactive intensity.

Second, levels of hyperphosphorylated tau pathology and numbers of extracellular, plaque-like Aβ deposits were determined by a counting method based on our previously established exclusion and inclusion criteria (Carroll et al., 2007; Rosario et al., 2006). Tau pathology was quantified by counting the combined number of AT8-immunoreactive neurons, as defined by immunoreactive staining throughout the cell body, in hippocampus and subiculum from 11–12 sections per brain (each separated by 280 μm). The number of extracellular plaque-like Aβ deposits was counted in a similar manner in hippocampus and subiculum from 11–12 sections per brain from Aβ-immunolabeled sections in which the size of the immunolabeled plaque was at least twice the size of a neuron cell body. Although plaque number is related to Aβ IHC load, and both measures are affected by threshold parameters, they represent different stages of pathology with the plaque stage representing later, more advanced pathology in 3xTg-AD mice (Oddo et al., 2003; Rosario et al., 2006).

Raw data from Aβ load data was analyzed by repeated measures ANOVA with the Wilks' lambda test. Raw data from Aβ load values, AT8 counts, and Aβ plaque counts were statistically analyzed by a one-way ANOVA, followed by between group comparisons using Fisher LSD. Effects with $p < 0.05$ were considered statistically significant.

Research Highlights

- Testosterone reduces Alzheimer-related pathology in a transgenic mouse model
- An androgen metabolite of testosterone reduces beta-amyloid but not tau pathology
- An estrogen metabolite of testosterone is effective in reducing tau pathology
- Testosterone uses both androgen and estrogen pathways to protect against Alzheimer

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Abbreviations

Aβ	β-amyloid
AD	Alzheimer's disease
APP	amyloid precursor protein
AR	androgen receptor
DHT	dihydrotestosterone
E_2	17β-estradiol
ER	estrogen receptor
GDX	gonadectomy
T	testosterone

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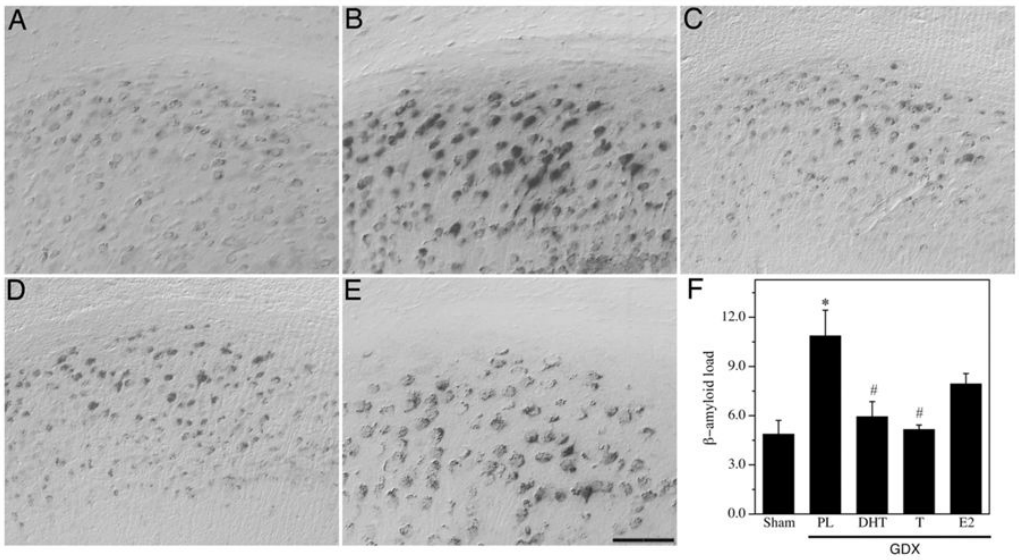

Fig. 1.

Hormone regulation of Aβ in subiculum of male 3xTg-AD mice. Representative high magnification photomicrographs show Aβ immunoreactivity in male 3xTg-AD mice in the following treatment groups: Sham GDX (A), GDX+PL (B), GDX+DHT (C), GDX+T (D), and GDX +E2 (E). Scale bar = 100μm. (F) Levels of Aβ immunoreactive load in subiculum were quantified across groups. Data show mean Aβ load values (± SEM). * Denotes $p < 0.05$ in comparison to Sham GDX (Sham) group, # denotes $p < 0.05$ versus GDX+PL group.

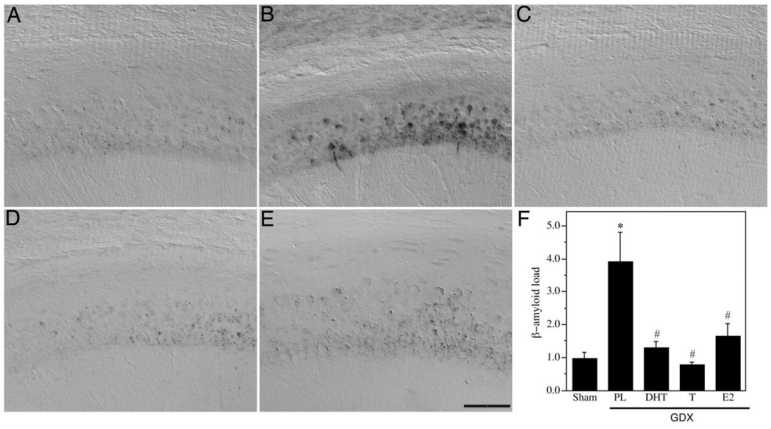

Fig. 2.

Hormone regulation of Aβ in hippocampus CA1 of male 3xTg-AD mice. Representative high magnification photomicrographs show Aβ immunoreactivity in male 3xTg-AD mice in the following treatment groups: Sham GDX (A), GDX+PL (B), GDX+DHT (C), GDX+T (D), and GDX +E2 (E). Scale bar = 100μm. (F) Levels of Aβ immunoreactive load in CA1 of hippocampus were quantified across groups. Data show mean Aβ load values (± SEM). * Denotes $p < 0.05$ in comparison to Sham GDX (Sham) group, # denotes $p < 0.05$ versus GDX+PL group.

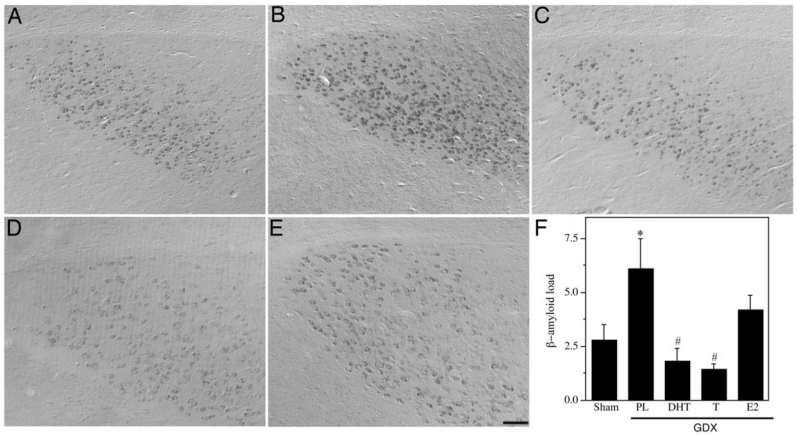

Fig. 3.

Hormone regulation of Aβ in amygdala of male 3xTg-AD mice. Representative high magnification photomicrographs show Aβ immunoreactivity in male 3xTg-AD mice in the following treatment groups: Sham GDX (A), GDX+PL (B), GDX+DHT (C), GDX+T (D), and GDX +E2 (E). Scale bar = 100μm. (F) Levels of Aβ immunoreactive load in amygdala were quantified across groups. Data show mean Aβ load values (± SEM). * Denotes $p < 0.05$ in comparison to Sham GDX (Sham) group, # denotes $p < 0.05$ versus GDX+PL group.

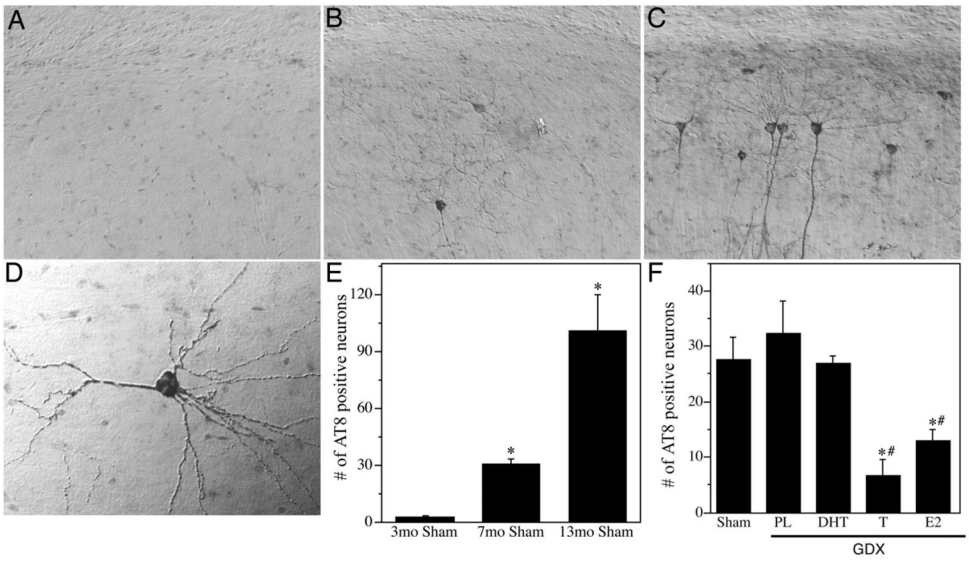

Fig. 4.

Age and regulation of tau hyperphosphorylation in male 3xTg-AD mice. (A–C) Representative photomicrographs show tau immunoreactivity with the phospho-specific antibody AT8 in hippocampus CA1 of Sham GDX male 3xTg-AD mice at (A) age 3 mo, (B) age 7 mo, and (C) age 13 mo old. Scale bar = 100 μm. (D) High magnification photomicrograph of a cell strongly immunoreactive for AT8 antibody. Scale bar = 25μm. (E) Levels of tau hyperphosphorylation were quantified by counts of cells strongly immunoreactive for AT8. Data show mean numbers (± SEM) of AT8-immunoreactive cells in Sham GDX males at ages 3 mo, 7 mo, and 13 mo. * Denotes $p < 0.05$ in comparison to 3 mo group. (F) Data show mean numbers (± SEM) of AT8-immunoreactive cells in Sham GDX (Sham), GDX+PL, GDX+DHT, GDX+T, and GDX+E2 mice at age 7 mo. * Denotes $p < 0.05$ in comparison to Sham group, and # denotes $p < 0.05$ in comparison to GDX+PL group.