Endocrine, *vol. 29, no. 2, 233–241, April 2006* 0969–711X/06/29:233–241/$30.00
ENDO (Online) ISSN 1559-0100

Androgens, Aging, and Alzheimer's Disease

Christian J. Pike,* Emily R. Rosario, and Thuy-Vi V. Nguyen

Andrus Gerontology Center, University of Southern California, Los Angeles, CA USA 90089-0191

Testosterone depletion is a normal consequence of aging in men that is associated with senescent effects in androgen-responsive tissues. We discuss new evidence that one consequence of testosterone depletion in men is an increased risk for the development of Alzheimer's disease (AD). Furthermore, we discuss two candidate mechanisms by which testosterone may affect AD pathogenesis. First, testosterone has been identified as an endogenous regulator of β-amyloid, a protein that abnormally accumulates in AD brain and is implicated as a causal factor in the disease. Second, findings from several different paradigms indicate that testosterone has both neurotrophic and neuroprotective functions. These new findings support the clinical evaluation of androgen-based therapies for the prevention and treatment of AD.

Key Words: Testosterone; Alzheimer's disease; β-amyloid; neuroprotection; dihydrotestosterone; luteinizing hormone.

Introduction

Advancing age is the most significant risk factor for developing Alzheimer's disease (AD); however, the age-related changes underlying this risk have yet to be fully elucidated. One well-defined age change that is associated with disease and dysfunction is depletion of sex steroid hormones. In women, the nearly complete loss of estrogen following menopause is linked to a range of disorders including AD *(1–4)*. Here we discuss recent evidence suggesting that a parallel relationship is evident in men. It is now well established that, as a normal consequence of aging, men experience a gradual but often robust decrease in androgen levels. Beginning in the third decade of life, circulating levels of total testosterone in men start to decrease at a rate of 0.2%–0.8% per year *(5–7)*. Because circulating levels of sex hormone binding globulin increase at a rate of 1.1%–1.6% per year *(5–10)*, the free testosterone levels decrease at a much higher rate (2%–3% per year) than does total testosterone *(5–7)*. The results of numerous cross-sectional *(6–8,11–20)* and longitudinal *(5,10,21,22)* studies yield the same finding: testosterone levels decrease with advancing age in men. Interestingly, recent data show that testosterone levels in brain tissue exhibit a similar age-related depletion *(23)*. The reasons for testosterone loss include both primary and secondary hypogonadism. That is, the testes are less able to produce testosterone, and the hypothalamic pituitary gonadal axis, which regulates testosterone levels, exhibits impaired responsiveness (for review see refs. *24–27*).

Accumulating evidence indicates that the consequences of normal, age-related testosterone depletion can be quite significant. Decreasing androgen levels are associated with impaired functioning and increased risk for disease in androgen-responsive tissues, including muscle *(28–32)*, bone *(33–35)*, and heart *(33–35)*. The constellation of senescent effects linked to male hypogonadism is increasingly recognized as a clinical syndrome termed androgen deficiency in aging males (ADAM). As an androgen-responsive organ, the brain is also vulnerable to age-related androgen loss. Neural manifestations of ADAM include disturbances in mood, cognition, and libido *(24,25,27,36)*. In this review, we discuss recent evidence that age-related androgen loss in men may also increase the risk of developing AD. Although the mechanism(s) by which androgen depletion promotes AD pathogenesis is not clear, two candidate pathways are considered: regulation of β-amyloid protein and neuroprotection.

Testosterone Loss Is a Risk Factor for Alzheimer's Disease

Recent evidence indicates that the senescent consequences of age-related testosterone depletion include increased risk for the development of AD. One of the earliest studies to investigate this issue found significantly lower total testosterone levels in serum from institutionalized aged men with dementia than in community-dwelling, age-matched controls *(37)*. Although interesting, the results of this study may have been affected by the fact that only the AD men were institutionalized, a condition that itself is associated with decreased testosterone *(38–40)*. For example, in comparison to community-dwelling healthy aged men, institutionalized aged men with neurologic disease had lower total and free testosterone; however, their low testosterone levels were not significantly different than institutionalized aged men lacking neurologic disease *(41)*. The first study to prop-

Received June 20, 2005; Revised October 24, 2005; Accepted October 24, 2005.

Author to whom all correspondence and reprint requests should be addressed: Christian J. Pike, Andrus Gerontology Center, University of Southern California, Los Angeles, CA USA 90089-0191. E-mail: cjpike@usc.edu

erly control for potential confounds while directly investigating the relationship between testosterone and AD was conducted by Hogervorst and colleagues *(42)*. They reported that in an English sample circulating levels of total testosterone were significantly lower in men with AD than in a control group of aged, nondemented men. Subsequent studies by this group confirmed and extended this important observation. In male AD cases with mean ages less than 80 yr, circulating levels of total *(43–45)* and free *(46)* testosterone were observed to be significantly lower than in aged male controls, independent of a variety of potential confounds including age and body mass index. More recently, other research groups have reported similar findings in studies of men from other ethnic groups. In a Japanese sample, Watanabe et al. *(47)* found that men with AD exhibited significantly lower total testosterone than healthy, non-demented men. In an Italian sample, Paoletti and colleagues *(48)* reported that men diagnosed with AD had significantly lower levels of free testosterone but not total testosterone in comparison to non-demented control cases *(48)*. Non-significant trends toward reduced total testosterone in AD vs healthy, aged men were reported in Swedish *(49)* and Australian *(50)* cohorts. In one recent study that failed to control for potential confounds and had a small sample size, testosterone levels were observed to be higher in AD cases as compared with controls *(51)*. Interestingly, recent data suggest that low testosterone may also be associated with age-related neurodegenerative conditions other than AD, including vascular dementia *(47)*, amyotrophic lateral sclerosis *(52)*, Huntington's disease *(53)*, and Parkinson's disease *(54)*. In contrast to the relationship between low testosterone and neurodegenerative disease in men, several recent studies found no evidence of altered testosterone levels in women with AD *(42,48,55,56)*.

One criticism of these studies is that they do not address whether low testosterone is a predecessor or a co-morbid characteristic of AD. That is, does testosterone depletion occur prior to and thus potentially contribute to AD pathogenesis or instead does it decrease as a consequence of disease progression? It is known that circulating levels of testosterone are significantly decreased by a number of disease conditions, including diabetes, alcoholism, kidney disease, liver disease, and others *(6,57–66)*. Furthermore, as men with AD increase in age their testosterone levels continue to decline, perhaps suggesting a relationship between advancing AD severity and decreasing testosterone levels *(54)*. However, two recent and complementary studies strongly suggest that testosterone depletion occurs prior to onset of AD and thus likely functions as a contributing causal factor in development of AD *(23,67)*. First, using blood samples collected over many years from the Baltimore Longitudinal Study of Aging, Moffat and colleagues confirmed prior results that circulating levels of free testosterone are significantly lower in men with AD even following adjustment for potential confounding factors *(67)*. Owing to the longitudinal nature of the study, the authors were able to assay testosterone levels at times prior to the clinical diagnosis of AD. Analysis of samples from both 5 and 10 yr prior to initial diagnosis revealed the same relationship of lower free testosterone in the men that later developed AD. Thus, these prospective data indicate that AD is preceded by depletion of circulating testosterone.

In a second study implicating testosterone depletion as a contributing rather than consequential factor in AD pathogenesis, Rosario and colleagues analyzed levels of testosterone in brain tissue from male AD and control cases *(23)*. Analysis of hormone levels in tissue has an important advantage in that tissue measurements represent the actual bioavailable hormone amount, which can differ from circulating levels *(27,68,69)*. In agreement with analyses of circulating testosterone *(42–44,46–48,67)*, Rosario et al. reported that testosterone levels in brain were significantly lower in men with AD than in non-diseased men *(23)*. Importantly, samples from selected cases were collected with short postmortem delays and neuropathologically characterized as either pure AD or lacking evidence of any neuropathology. In a separate study with a small sample size and quite long postmortem delays, Twist and colleagues observed a non-significant trend of reduced testosterone levels in brain samples from male AD cases vs controls *(70)*. To address whether the observed low testosterone in AD cases may contribute to disease development, Rosario et al. evaluated a third subject group that was cognitively normal but exhibited mild neuropathology consistent with the initial stages of AD. Brain levels of testosterone in the mild neuropathology group were very similar to the AD group but significantly lower than the controls. As with the study by Moffat et al. *(67)*, these data suggest that testosterone reduction occurs prior to disease onset and thus is a risk factor for development of AD.

One factor that may influence the strength of the testosterone/AD relationship is age. In most of the studies discussed thus far, the mean age of the men under study has been in the low to mid 70s. Recently, Hogervorst et al. reported that in younger, aged men (mean age 66 yr) AD was characterized by significantly lower levels of free testosterone with no significant difference in either total testosterone or the gonadotropins (discussed further below) luteinizing hormone (LH) and follicle stimulating hormone (FSH) *(46)*. However, in relatively older, aged men (mean age 80 yr), AD was characterized by no change in free testosterone, a modest reduction in total testosterone, and significant elevations in both LH and FSH. Similarly, we have recently extended our studies of brain levels of testosterone to include older men. Although we found significantly lower brain levels of testosterone in AD men younger than 80 yr (mean age 72 yr) in comparison to age-matched controls (mean age 71 yr) *(23)*, similar experiments with men over

the age of 80 (mean age = 86) showed no significant relationship. We suspect that, as has been proposed for estrogen responsiveness in aging women *(71)*, there may be a critical window during which the aging brain retains androgen responsiveness but after which it exhibits relative functional indifference to testosterone depletion.

Accumulating data suggest that the testosterone/AD relationship may also involve an interaction between testosterone and another key risk factor for AD, the ε4 allele of apolipoprotein E (apoE4) *(72)*. ApoE4 appears to be the primary genetic risk factor for the development of late-onset AD (for reviews see *(73,74)*. In a study by Hogervorst et al., normal, non-demented men with an apoE4 allele were found to have significantly lower circulating levels of testosterone than men lacking an apoE4 allele *(44)*. Interestingly, a non-significant trend toward higher testosterone levels was observed in men with an apoE2 allele, which is associated with reduced risk for AD *(75)*. In rodent studies implicating an interaction between androgen levels and apoE4, Raber et al. reported that female but not male mice engineered to express human apoE4 exhibit deficits in learning and memory *(76)*. Cognitive deficits in female apoE4 mice were attenuated by treatment with either testosterone or DHT. In male apoE4 mice, learning deficits were induced by treatment with the androgen receptor antagonist flutamide. Furthermore, apoE4 was associated with reduced levels of androgen receptor *(76)*. In a separate rodent study, male apoE4 mice exhibited greater release of potentially harmful, pro-inflammatory molecules than male apoE3 mice *(77)*. Together, these and other new data suggest that low testosterone may interact with apoE4 and perhaps other risk factors in promoting AD pathogenesis.

Androgens Regulate Levels of β-Amyloid Protein

If testosterone depletion does indeed promote the development of AD, understanding the underlying mechanism(s) of this relationship will be critical for the design and potential use of androgen replacement therapies in the prevention and or treatment of AD. An obvious candidate that deserves consideration is β-amyloid (Aβ), a normal protein whose abnormal accumulation is widely implicated as the initiating causal factor in the degenerative cascades of AD. According to the generally but not universally embraced Aβ cascade hypothesis *(78,79)*, conditions that result in the neural accumulation of Aβ— by either increasing Aβ production or decreasing Aβ clearance—will promote the development of AD. Because many of the established genetic and environmental risk factors of AD are known to regulate levels of Aβ, the possible regulatory relationship between testosterone and Aβ should be evaluated.

Prior studies established that estrogen decreases levels of Aβ in cell culture paradigms *(80–85)*, wild-type *(86)* and transgenic rodents *(87,88)*, and perhaps even in women *(89, 90)*. The first investigation of possible testosterone regulation of Aβ was reported by Gouras et al. *(91)*. Using cultures of both murine neuroblastoma N2a cells and rat primary cerebrocortical neurons, they found that estrogen and testosterone had significant effects on Aβ generation. The approx 4 kDa Aβ is derived from proteolytic processing of its much larger parent protein, amyloid precursor protein (APP) (for review see ref. *92*). The proteolytic enzyme α-secretase cleaves APP at residue 17 in the 40–42 amino acid Aβ, thereby preventing generation of full length Aβ. This non-amyloidogenic processing of APP yields a product termed soluble APPα (sAPPα), which serves as a useful marker of APP metabolism and Aβ generation. Gouras et al. found that treating cultures for an extended period (4–14 d) with supraphysiological concentrations (200 n*M*, 1 μM) of either estrogen or testosterone resulted in significantly decreased levels of Aβ. In addition, levels of sAPPα were elevated while total APP levels were unchanged, suggesting hormone-induced promotion of non-amyloidogenic APP processing. Although certainly compelling, it is unclear whether these data represent a novel androgen action or, conversely, an indirect activation of an established estrogen effect. Several points are relevant here: (i) estrogen decreases Aβ levels by a mechanism that involves, at least in part, altered APP processing and or trafficking *(82,83)*, (ii) neurons are known to contain the enzyme aromatase that converts testosterone to estrogen *(93,94)*, and (iii) the high testosterone concentrations and extended exposure times used by Gouras et al. are consistent with the possible in vitro aromatization of testosterone to estrogen. In agreement with this possibility are the data of Goodenough et al. *(95)*. Using an immortalized rat hypothalamic cell line, they found that a physiological concentration of testosterone (10 n*M*) rapidly increased secreted sAPPα levels but did not affect total APP levels, indicating testosterone-induced alteration of endogenous APP processing to a non-amyloidogenic pathway. Notably, testosterone regulation of APP processing was completely blocked by an aromatase inhibitor, suggesting that testosterone regulation of Aβ may occur largely via estrogen pathways.

A more recent study indicates that brain levels of Aβ are reduced by androgen but not estrogen pathways in adult male rats. To investigate this issue, Ramsden et al. first determined how brain levels of soluble Aβ were affected by depletion of sex steroid hormones resulting from orchidectomy (ORX) *(96)*. They found that Aβ levels in hemi-brain homogenates were significantly increased in ORX vs sham male rats, demonstrating that testosterone loss elevates Aβ levels and thus suggesting that testosterone functions as an endogenous regulator of brain Aβ. To determine whether this effect involved androgen or estrogen pathways, they treated additional groups of ORX rats with either estrogen or DHT, a potent and non-aromatizable androgen. Four-week treatment with DHT reduced the ORX-induced increase in Aβ

to levels even lower than those observed in gonadally intact control animals *(96)*. Interestingly and perhaps unexpectedly, estrogen treatment in ORX rats did not significantly alter Aβ levels. There were no obvious changes in levels of either full length APP or sAPPα across the different treatment groups. The apparent lack of an androgen effect on APP processing in vivo appears to contradict cell culture data *(91,95)*, thus raising the issue of how androgens regulate Aβ levels. Notably, prior in vivo studies with estrogen *(86,87)* and with phorbol ester *(84)* have yielded similar discordance with in vitro predictions that sAPPα should be elevated in parallel to Aβ reduction. Although the relevant mechanism(s) remain to be elucidated, the data clearly demonstrate that androgen status is a potent predictor and likely endogenous regulator of Aβ.

Consistent with the predictions of in vitro and in vivo studies, initial evidence suggests that androgens regulate Aβ levels in aged men. In a small study by Gandy et al., six aged men undergoing treatment for prostate cancer were assayed for circulating levels of testosterone, 17β-estradiol, and Aβ before and during application of anti-androgen therapy consisting of both flutamide (an AR antagonist) and leuprorelin acetate (a GnRH agonist). Within 4 wk of initiating treatment, all men showed robust depletion of both estrogen and testosterone as well as obvious increases in Aβ levels *(97)*. Using a similar experimental design, Almeida et al. reported on a larger sample of 40 men who underwent a 36-wk period of anti-androgen therapy and were followed for another 18 wk after treatment cessation *(98)*. In agreement with the Gandy et al. study *(97)*, they found that anti-androgen therapy resulted in depletion of estrogen and testosterone, both of which were significantly and inversely correlated with plasma levels of Aβ. After anti-androgen therapy was discontinued, there was a non-significant trend toward reversal of these relationships, with hormone levels rising and Aβ falling *(98)*. Interestingly, anti-androgen therapy was associated with increased measures of depression and anxiety, whereas cessation of therapy resulted in indications of cognitive improvement *(98)*. In a recent study of men with either subjective memory loss or diagnosed dementia, circulating levels of total and free testosterone were again inversely correlated with plasma Aβ *(99)*. Together, these initial studies are consistent in observing a significant relationship between low testosterone levels and elevated Aβ levels. Less certain is to what extent the relationship reflects androgen vs estrogen contributions. In the Gandy et al. *(97)* and Almeida et al. *(98)* studies, the concomitant loss of both estrogen and testosterone by anti-androgen therapy precludes unambiguous determination of the hormone(s) responsible for Aβ regulation. The Gillet et al. study, which did not involve anti-androgen therapy, failed to observe a significant relationship between estrogen and Aβ levels. Also suggestive of a predominantly direct androgen effect in hormone regulation of Aβ in males is the rodent study by Ramsden et al. in which estrogen treatment of ORX males did not reduce brain levels of Aβ *(96)*.

Androgens Regulate Neuron Viability

In addition to beneficially regulating Aβ levels, another androgen function that may contribute to its protective role against development of AD is promotion of neuron viability. Consistent with an androgen role in regulation of neuron viability are neurodevelopment studies in which testosterone, either directly through androgen pathways or indirectly (via aromatization) through estrogen pathways, regulates neuron number in specific sexually dimorphic brain regions *(100–103)*. In addition, testosterone—again both directly and indirectly—exerts a variety of neurotrophic effects, including neuronal differentiation *(104,105)*, neurite outgrowth *(106–109)*, hippocampal spine growth *(110,111)*, hippocampal excitability *(112,113)*, and morphological and structural integrity of motor *(114–117)* and autonomic *(118,119)* neurons. Furthermore, testosterone can influence glial functions in ways that may improve neuronal survival *(77,120–125)*.

One well-described neuroprotective action of testosterone is its promotion of recovery in peripheral motoneurons following injury (for review see ref. *126*). In a common paradigm, cranial nerves (e.g., facial, hypoglossal) in male or female rodents are axotomized and nerve regeneration and or motoneuron survival evaluated several weeks later under varying hormone treatment conditions. Testosterone treatment following axotomy can accelerate the rate of nerve regeneration *(126–131)* and, in young animals, attenuate motoneuron loss *(132–134)*. In the case of motoneuron viability, early studies indicated that only long-term hormone treatment is correlated with increased survival *(133)*, implicating target-derived trophic factors from muscle in the protective mechanism *(135)*. However, in very recent work, even relatively short-term treatment with testosterone, DHT, or estrogen can reduce loss of facial motoneurons *(134)*, perhaps indicating more direct neuroprotective mechanisms. In nerve regeneration, it appears that endogenous androgen levels may not be a crucial factor as rates of regeneration observed following facial nerve axotomy are similar in ORX and gonadally intact adult male hamsters *(130)*. As observed with neuroprotection *(134)*, testosterone, DHT, and estrogen are equally effective in promoting nerve regeneration in this model *(131)*.

More relevant to this discussion is how testosterone affects neuron loss in brain regions implicated in age-related neurodegenerative disorders such as AD. In the hippocampus, which is both AR-rich *(136,137)* and severely affected by AD neuropathology *(138,139)*, androgens are neuroprotective. In adult male rats, Ramsden et al. found that androgen depletion resulting from ORX significantly increased loss of hippocampal pyramidal and hilar neurons following lesion with the excitotoxin kainate *(140)*. These data suggest that androgens function as endogenous regulators of hippocampal neuron viability. Furthermore, 2-wk treatment of ORX animals with the non-aromatizable androgen DHT

significantly protected against kainate-induced neuron loss without altering latency or severity of kainate-induced seizure behaviors *(140)*. These data suggest an androgen neuroprotective pathway that is independent of both estrogen action and seizure inhibition.

Interestingly, related studies by other groups show that testosterone can also exert neuroprotection by the two pathways not implicated by the Ramsden et al. study: aromatization to estrogen *(141)* and seizure inhibition *(142–145)*. First, in agreement with Ramsden et al. *(140)*, Azcoitia et al. observed that gonadally intact but not ORX adult male rodents were resistant to neuron loss in the hilus of the dentate gyrus induced by excitotoxins *(141)*. Acute treatment of ORX animals with either testosterone or estradiol was neuroprotective. However, acute treatment with the non-aromatizable androgen DHT did not afford protection, and testosterone protection was blocked by an aromatase inhibitor, indicating that aromatization of testosterone to estradiol may be responsible for the protective action of testosterone in this experimental model *(141)*. The absence of direct androgen protection in the study by Azcoitia et al. *(141)* may be due to timing of hormone administration. In the Ramsden et al. paradigm, DHT was continually present for 2 wk before kainate lesion, whereas Azcoitia et al. delivered the hormone immediately following kainate injection. Since DHT was restored after 3 wk of hormone withdrawal, it is unlikely that the AR and signal transduction mechanisms present in the Ramsden et al. *(140)* animals were fully activated in those used by Azcoitia et al. *(141)*. In related seizure paradigms, Frye and colleagues observed that testosterone reduced hippocampal injury by a mechanism involving inhibition of seizure activity *(144,145)*. Testosterone is metabolized by 5α-reductase into the potent AR agonist DHT, which is then reversibly converted to 5α-androstane-3α,17α-diol (3α-diol) by the oxioreductase 3α-hydroxysteroid dehydrogenase. The DHT metabolite 3α-diol—like the closely related progesterone metabolite allopregnanolone—is known to act as a potent modulator of $GABA_A$ receptor, increasing chloride conductances activated by GABA, decreasing excitatory signaling and thereby antagonizing seizure activity *(142,143)*. Together, these studies indicate that androgens are neuroprotective in hippocampus, a brain region with abundant AR *(136,137)*. Notably, at least three different mechanisms of neuroprotection are implicated: activation of androgen neuroprotective pathways *(140)*, aromatization to estradiol *(141)*, and anxiolytic effects of the DHT metabolite 3α-diol *(142)*.

In contrast to these examples of testosterone-mediated neuroprotection, in some disease-related lesion models testosterone appears to lack beneficial effects on neuron survival. For example, Dluzen et al. used an ovariectomy (OVX) model in female mice to demonstrate neuroprotective actions of estrogen against nigrostriatal dopaminergic toxicity induced by the toxin methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) *(146)*. Interestingly, using either the same MPTP model *(147)* or a related methamphetamine paradigm *(148)*, Dluzen et al. found that testosterone failed to protect ORX male mice against dopaminergic toxicity. Similar results of estrogen protection but an absence of protection by either testosterone or DHT were also reported by Ekue et al. *(149)*. Perhaps surprisingly, there is evidence suggesting that testosterone can actually exacerbate some forms of neural injury. Using striatal injection of the mitochondrial toxin 3-nitroproprionic acid in adult rats, Nishino et al. found that gonadally intact and ORX exhibited similar lesions, suggesting endogenous androgens do not regulate vulnerability to this insult. However, in OVX female rats, testosterone increased and estrogen reduced lesion severity *(150)*. These results suggest that androgens and estrogen may work through opposing strategies in modulating striatal damage. Similarly, in a middle cerebral artery occlusion model of ischemia-reperfusion, Simpkins and colleagues found that ORX-induced androgen depletion reduced injury in male rats *(151,152)*. Furthermore, hormone replacement of ORX rats with testosterone increased neural injury whereas estrogen protected *(152)*. In contrast to the observations with motoneurons, these lesion studies indicate that, in response to at least some insults, testosterone can fail to exert neuroprotection and may even potentiate neural injury. Future research must address the differential ability of testosterone to regulate neuronal viability across different types of neural injury. Our recent studies indicate that testosterone protects neurons from insults that induce an apoptotic form of cell death but not from insults that cause necrosis (Nguyen and Pike, unpublished observations).

In addition to in vivo demonstration of androgen neuroprotection, several studies using primary neuron cultures indicate that testosterone and related androgens are directly neuroprotective. For example, testosterone protects against cell death induced by serum deprivation in cultures of both motor neurons *(115)* and human brain neurons *(153)*. Furthermore, testosterone significantly reduces neuron death caused by exposure to toxins, including pro-oxidants *(154)* and extracellularly *(155)* and intracellularly *(156)* applied Aβ. Interestingly, testosterone can also inhibit phosphorylation of tau *(157)*, a neuroprotective effect potentially relevant to AD. The neuroprotective mechanism appears to involve androgen, not estrogen, pathways. For example, in the study by Pike, testosterone neuroprotection against Aβ toxicity was mimicked by the non-aromatizable DHT and was not inhibited by the estrogen-receptor antagonist droloxifene *(155)*. Further supporting direct involvement of androgen pathways is the observation that neuroprotection can be inhibited by the anti-androgen flutamide *(153,154, 156)*. In the study by Hammond et al., the aromatase inhibitor 4-androsten-4-OL-3,17-dione failed to block testosterone-mediated neuroprotection and the non-aromatizable androgen mibolerone was protective *(153)*. In a recent study by Nguyen et al., androgen neuroprotection is observed in a neural cell line stably transfected with AR, but not in either

wild-type or empty vector-transfected cells *(158)*. Finally, the DHT metabolite 3α-diol does not affect neuron viability in the same paradigm in which both testosterone and DHT are neuroprotective *(155)*. Together, these in vitro studies support AR-mediated androgen pathways in the mechanism of androgen neuroprotection.

Although neuron culture studies have clearly established a role for AR in androgen neuroprotection, the downstream mechanism(s) of protection are still being defined. Results from Ahlbom et al. suggest that an antioxidant defense mechanism may be involved because testosterone treatment resulted in a twofold increase in activity of the antioxidant enzyme catalase *(154)*. Recent data from Zhang et al. suggest a second potential mechanism of androgen neuroprotection: induction of heat shock proteins *(156)*. They observed that androgen neuroprotection was associated with increased levels of heat shock protein 70 *(156)*, a factor known to participate in neuroprotective responses *(159)*. Most recently, Nguyen et al. reported that androgen neuroprotection in both cultured hippocampal neurons and an AR-transfected neural cell line involves AR-dependent activation of a mitogen-activated protein kinase (MAPK)/extracellular signal regulated kinase (ERK) pathway *(158)*. They observed that testosterone and DHT rapidly and transiently induce a nongenomic MAPK signaling pathway involving activation of ERK, followed by activation of p90 kDa ribosomal S6 kinase (Rsk), which in turn phosphorylates and thereby inactivates the pro-apoptotic protein Bad. Interruption of this signaling pathway at either the ERK or Rsk steps prevented Bad phosphorylation and blocked androgen neuroprotection *(158)*. These studies suggest that androgen activation of AR signaling in neurons likely results in several genomic and nongenomic changes that yield net increases in neuron viability and thus increase neuronal resistance to toxic insults.

What Is the Role of Gonadotropins in Alzheimer's Disease?

As discussed above, normal male aging is associated with age-related declines in total and bioavailable levels of androgens as well as increased levels of the gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH). Because testosterone levels in men are regulated by LH actions in a negative feedback loop via the hypothalamic–pituitary–gonadal axis, it is possible that at least some of the effects attributed to low testosterone are actually due to high LH. That is, perhaps it is the age-related rise in the LH rather than, or in addition to, the decline in androgens that results in increased risk of AD, accumulation of Aβ, and impaired neuron survival *(160)*. In support of this possibility, Bowen et al. reported that men with AD exhibit significantly higher levels of LH than non-demented, aged controls *(37)*. A second study by this group failed to reproduce the finding of higher LH in men with AD *(161)*. It may be that significant elevations in circulating LH levels do not occur until a relatively late stage in normal male aging *(21)*, and thus the relationship between elevated LH and diagnosis of AD may be most apparent in very old age *(43)*. Consistent with this possibility are recent findings of Hogervorst et al. *(46)*. When they stratified aged men with or without AD into relatively younger (mean age approx 65 yr) and older (mean age approx 80 yr) age groups, they observed a significant relationship between LH and AD status only in the older men *(46)*. In contrast, significantly lower free testosterone was specifically observed in the younger AD group *(46)*. Perhaps both low testosterone and high LH act as independent risk factors for development of AD and do so by separate mechanisms.

It is reasonable to consider that LH may contribute to our findings in animal models in which ORX results in increased Aβ levels *(96)* and decreased neuron survival *(140)*. Both effects are reversed by DHT treatment of GDX males *(96, 140)*. As a consequence of depleting endogenous androgens, ORX also causes robust elevation of LH (via HPG feedback loops). DHT replacement in ORX animals restores androgen action and stabilizes LH levels. So, is LH or DHT responsible for mediating effects on Aβ and neuron viability? Interestingly, LH levels are modulated by both androgen *(162,163)* and estrogen *(164)* actions. Thus, estrogen replacement in ORX male animals can restore LH levels to normal levels. In our rodent paradigms, we observe that although estrogen reverses ORX-induced elevations in LH, estrogen neither provides neuroprotection (unpublished observations) nor reduces Aβ levels *(96)*. Furthermore, in their study of aged men with cognitive deficits, Gillet et al. found a significant inverse relationship between circulating levels of Aβ and both total and free testosterone, but no relationship between Aβ and LH *(99)*. These data indicate a disconnect between LH levels and the two key androgen actions we hypothesize are associated with AD pathogenesis. Note, however, that a recent study by Bowen et al. shows a relationship between LH and nonamyloidogenic processing of APP *(165)*, perhaps suggesting independent mechanisms by which LH and testosterone can regulate pathways implicated in AD pathogenesis.

Conclusions

In this review, we have discussed accumulating evidence that low testosterone is a risk factor for the development of AD in men. Progressive loss of testosterone is a normal consequence of male aging that is associated with senescent effects in androgen-responsive tissues, including brain. Recent cross-sectional and longitudinal data link this age-related testosterone depletion with increased risk for AD. We hypothesize that this increased risk of AD is due at least in part to the significant role of androgens as endogenous regulators of two key neural events: Aβ accumulation and neuroprotection. We present evidence that androgens, through both direct androgen pathways and via aromatization to estro-

gen, function to decrease levels of Aβ and increase neuronal resilience to toxic insults. We predict that the loss of testosterone with advancing age creates a more hostile neural environment that promotes accumulation of toxic Aβ while leaving neurons less able to survive the insult. Elucidating the cellular and molecular mechanisms of these androgen actions will promote the targeted development of androgen therapies for use in the prevention and treatment of AD and related neurodegenerative disorders.

Acknowledgments

This work was supported by grants from the Alzheimer's Association (IIRG-04-1274) and the NIA (AG23739).

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