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Sex hormones, aging, and Alzheimer's disease

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1. ABSTRACT

A promising strategy to delay and perhaps prevent Alzheimer's disease (AD) is to identify the age-related changes that put the brain at risk for the disease. A significant normal age change known to result in tissue-specific dysfunction is the depletion of sex hormones. In women, menopause results in a relatively rapid loss of estradiol and progesterone. In men, aging is associated with a comparatively gradual yet significant decrease in testosterone. We review a broad literature that indicates age-related losses of estrogens in women and testosterone in men are risk factors for AD. Both estrogens and androgens exert a wide range of protective actions that improve multiple aspects of neural health, suggesting that hormone therapies have the potential to combat AD pathogenesis. However, translation of experimental findings into effective therapies has proven challenging. One emerging treatment option is the development of novel hormone mimetics termed selective estrogen and androgen receptor modulators. Continued research of sex hormones and their roles in the aging brain is expected to yield valuable approaches to reducing the risk of AD.

2. INTRODUCTION

Increasing age is the most significant risk factor for the development of Alzheimer's disease (AD) (1-3). Even in persons with autosomal dominant mutations and genetic risk factors for AD, the disease develops during middle or advanced ages. Although the factors associated with normal aging that contribute to AD pathogenesis remain to be clearly determined, their identification promises significant insight into the development and perhaps prevention of the disease. In this review, we discuss evidence suggesting that the normal age-related depletion of sex steroid hormones represents an important age-related AD risk factor. The literature indicates that both the relatively abrupt loss of estrogen and progesterone at menopause in women and the more gradual decrease in testosterone in aging men are AD risk factors. As such, therapeutic strategies that counteract age-related depletion of sex steroid hormones may offer significant protection from the development and perhaps treatment of AD.

2.1. Aging and loss of sex hormones

Depletion of sex steroid hormones is an important consequence of normal aging that is associated

with vulnerability to disease in hormone-responsive tissues, including the brain (4-13). Following menopause, women experience relatively rapid loss of the ovarian sex hormones, 17beta-estradiol (E2) and progesterone (P4). Men also experience a significant age-related decrease in circulating testosterone levels, known as androgen deficiency in aging males (ADAM) (4). However, in contrast to menopause, ADAM is not necessarily coupled with the loss of reproductive function and hormonal changes are gradual, with bio-available testosterone levels declining 2-3% annually from approximately 30 years of age (14-16). A high level of individual variation is observed in the extent of ADAM (17, 18) and consequently, there is variability in the severity of clinical presentation, which includes reduced muscle and bone mass, increased fat, lethargy, depression and decreased libido (19-23).

While age related decline in circulating levels of gonadally produced sex steroid hormones has been well characterized, brain levels of hormones can significantly differ from circulating levels due to sequestration by sex hormone binding globulin, the presence of brain steroid converting enzymes, and neurosteroidogenesis (24-27). Few studies have addressed the effects of aging on brain hormone levels. However, female brain levels of E2 have been found to qualitatively mirror circulating E2 levels, with significant declines observed in the brains of postmenopausal women compared to premenopausal women (28), but with very little additional decrease with age after menopause (27, 28). Age-related declines in brain testosterone levels in men have been described, with brain testosterone levels depleted to very low levels by 80 years of age (27, 29). Interestingly, men show reduced but still significant levels of circulating testosterone even at advanced age (5, 16, 30). Further, while circulating levels of the potent androgenic metabolite of testosterone, dihydrotestosterone (DHT), do not appear to change with age (5), age-related declines in brain levels of DHT have been observed in both male rodents (31) and men (27).

2.2. Age-related sex hormone loss and AD risk

If age-related loss of sex steroid hormones is a contributing factor to AD, then it follows that the relatively sudden and extensive loss of E2 and P4 at menopause would result in women having greater vulnerability to AD than men. In fact, like several diseases, AD is characterized by an increased prevalence in women (32-38). Although increased lifespan in women complicates the interpretation of sex-differences in AD prevalence, incidence studies also demonstrate that women are at increased risk of AD (39-50). Further, more severe cognitive deficits and beta-amyloid (Abeta) neuropathology have been reported in women in comparison to men (51-54), although some studies have reported increased tau pathology in men (55, 56).

Sex differences in AD pathology have also been reported in several transgenic mouse models, with increased Abeta accumulation reported in female compared to age-matched male Tg2576 (57, 58), APPswe/PS1 (59, 60) and 3xTgAD transgenic mice (61, 62). Increased vulnerability of the female brain to AD has been primarily attributed to the loss of the neuroprotective sex steroid hormones following menopause, and much evidence from research in animal models and hormone therapy studies supports this notion. However, a recent study provides evidence that the developmental effects of the sex hormones (63) may also influence susceptibility to AD (61). Neonatal male 3xTgAD mice that were demasculinized with the androgen receptor antagonist flutamide exhibited a more female-like pattern of pathology with region-specific increases in Abeta accumulation. Conversely, female 3xTgAD mice defeminized by transient neonatal testosterone treatment showed regional reductions of Abeta accumulation (61). These findings suggest that sex steroid hormones may affect AD risk as a result of both organizational actions during development and loss of activational effects during aging.

Aside from sex differences, comparison of hormone levels demonstrate that age-related depletion of sex steroid hormones is linked with increased risk of AD in both women and men. Multiple studies have described a relationship between AD and low circulating levels of sex steroids - E2 in women and testosterone in men (64-71). For example, lower plasma 17beta-estradiol (E2) levels have been observed in women with AD compared to age-matched controls (69). Meanwhile, levels of both total (65, 72) and free plasma testosterone have been observed in men with AD compared to both vascular dementia sufferers (68) and age-matched controls (64, 67, 73, 74). Similarly, assessments of sex steroid hormone levels in the brain have demonstrated depleted testosterone levels observed in male AD brains, and depleted estrone and estradiol in female brain compared to age-matched cognitively normal controls (27, 29, 75, 76). Further, recent evidence suggests synthesis of sex hormones in the brain may also be affected, with altered levels of neurosteroidogenic enzymes observed in AD brains (77, 78).

Importantly, additional evidence suggests that hormone depletion occurs prior to the onset of AD and thus likely contributes to rather than results from the disease process. For example in a longitudinal study of aging men, the relationship between low testosterone and increased risk of AD was present 10 years prior to diagnosis of dementia (73). Further, in comparison to neuropathologically normal men, brain levels of testosterone are significantly lower not only in men with advanced AD but also in men exhibiting mild, AD-related neuropathological changes (29). Interestingly, an emerging literature suggests the possibility that AD pathology may negatively feedback on steroid levels by inhibiting neurosteroidogenesis. Although the regulation of endogenous brain steroid hormone production is incompletely understood, impaired neurosteroidogenesis has been observed in cell lines treated with Abeta and oxidative stress (79, 80), suggesting that depleted brain levels of the sex hormones may promote susceptibility to AD pathogenesis, which in turn could further reduce brain levels of neuroprotective sex hormones.

In AD, not only are brain levels of the sex hormones altered, but brain responsiveness may also be impaired as a result of altered sex hormone receptor levels

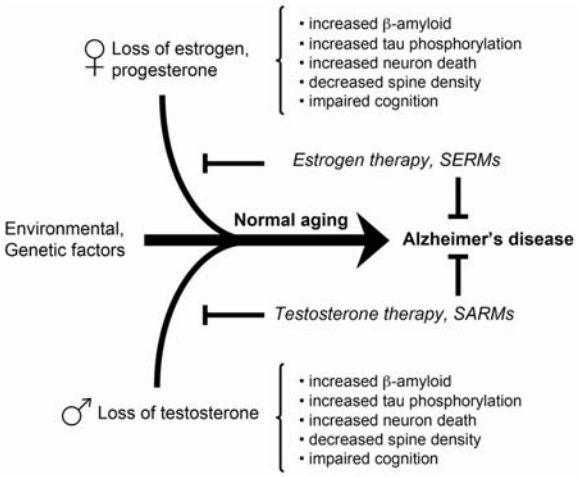

Figure 1. Interactions between age-related loss of sex hormones and risk of Alzheimer's disease. AD pathogenesis is a multifactorial process. Lifetime exposure to a combination of identified genetic and environmental risk factors interacts with numerous normal age changes to cooperatively promote the development of AD. One important normal age change that is linked to dysfunction and disease in many tissues is loss of sex hormones, estrogens in women and testosterone in men. Age-related depletion of estrogens and testosterone may be particularly significant to development of AD since these sex hormones are established regulators of several events implicated in the disease, including beta-amyloid accumulation, tau phosphorylation, neuronal death and decreased spine density. Early intervention using estrogen- and testosterone-based therapies or synthetic hormone mimetics termed estrogen receptor modulators (SERMs) and androgen receptor modulators (SARMs) may restore lost protective functions and prevent AD.

and distribution. Increased immunoreactivity of the estrogen receptors (ER) ERalpha (81) and ERbeta has been observed in the hippocampus of AD brains (82) and altered cellular localization of ER may also be associated with AD in the hippocampus (83) and hypothalamus (84). Polymorphisms of ERalpha have been linked to both familial and late-onset AD in multiple studies (85-92) and recently, reduced alternate splicing of ERalpha has been reported female AD brains (93). Although alternations in expression and distribution of AR in AD are comparatively unexplored, a polymorphism of the androgen receptor has also been associated with AD in men (94).

3. SEX HORMONES REDUCE BETA-AMYLOID LEVELS

If, as available evidence suggests, age-related loss of sex hormones increases the risk of AD, then a critical question is what hormone action(s) are most important to AD pathogenesis. Both estrogens and androgens exert numerous beneficial and protective actions in brain that have potential relevance to AD (Figure 1). As reviewed elsewhere, estrogens and androgens increase spine density and facilitate synaptic plasticity (95-99) and improve select aspects of cognition (45, 100-107). Also, estrogens and androgens are potent regulators of neuron viability, protecting neurons against a range of toxic insults including those implicated in AD (108-112).

Particularly relevant to a protective role against AD, sex hormones are implicated in reducing levels of Abeta, the protein widely implicated as the key initiator of AD pathogenesis. Human studies have associated depleted levels of E2 and testosterone with elevations in neural and plasma Abeta levels. For example, elevated Abeta levels are observed in the cerebrospinal fluid of women with low E2 (113). Further, a preliminary study in postmenopausal women with AD reported that estrogen-based hormone therapy (HT) was associated with lower plasma Abeta40 levels (114). In men, depleted circulating testosterone levels are associated with elevated Abeta levels in both cognitively normal (115, 116) and memory-impaired men (117). Testosterone depletion induced via chemical castration resulted in a corresponding increase in plasma Abeta levels in prostate cancer patients (115, 116). Testosterone levels have also been found to negatively correlate with soluble Abeta levels in brains from aged men (27, 29).

In animal studies, manipulation of E2 and testosterone through gonadectomy and hormone supplementation has also been found to significantly affect Abeta accumulation. Estrogen depletion resulting from ovariectomy (OVX) increases brain Abeta levels in many wild-type rodents and transgenic models of AD, including guinea pigs (118), APP (Tg2575) (119), APPswe (120), CRND8 (121), APP/PS1 (119, 122), and 3xTg-AD mice (123, 124), an effect that is partially reversed with E2 supplementation. However, in some animal models, OVX and E2 treatments do not significantly alter Abeta levels (76, 125-129). These discrepancies may reflect experimental differences in timing and dosing of hormone manipulations, or differences in Abeta quantification techniques, since different techniques preferentially detect different pools of Abeta (e.g. soluble vs. insoluble). Strain differences in brain levels of the sex hormones (130) may also contribute to the discrepancies in the effect of OVX on Abeta levels, since in some animal models OVX may be insufficient to induce brain E2 deficiency (76).

Compared to experimental studies in female animals, the effects of castration and testosterone supplementation on Abeta levels in male animals have been more consistent. Castration results in nearly complete loss of endogenous testosterone and corresponding elevations in Abeta in guinea pigs (131), rats (132), and 3xTg-AD mice (133). Because testosterone is a prohormone that is enzymatically converted within tissues to both the active androgen dihydrotestosterone (DHT) and the estrogen E2, there may be contributions from estrogens and androgens to Abeta regulation. In male rats, elevated levels of Abeta induced by castration were prevented by supplementation with DHT but not E2, suggesting a prominent role of androgen pathways (132). Similarly, preventing testosterone conversion to E2 by genetically limiting aromatase activity resulted in elevated testosterone levels,

low E2 levels, and reduced Abeta accumulation in male APP23 mice (134). However, in castrated male 3xTg-AD mice, Abeta burden was reduced not only by testosterone and DHT, but also by E2, suggesting that both androgens and estrogens can reduce Abeta in male brain (135).

3.1. Sex hormones regulate beta-amyloid production

The mechanisms by which estrogens and androgens regulate Abeta have yet to be fully elucidated, although both types of sex hormones have been implicated in regulating the production and clearance of Abeta. Production of Abeta results from the proteolytic cleavage of its parent protein, the amyloid precursor protein (APP). The majority of APP is metabolized by two competing pathways, the amyloidogenic and non-amyloidogenic pathways. In the amyloidogenic pathway, APP is sequentially cleaved by beta-secretase (BACE) and gamma-secretase, liberating Abeta peptides that largely occur in two species that are 40 and 42 amino acids in length. In the non-amyloidogenic pathway, APP is cleaved within the Abeta domain by alpha-secretase, preventing formation of full-length Abeta peptide, but releasing a soluble, protective form of APP termed APPalpha (136, 137).

Cell culture studies indicate that both E2 and testosterone may promote APP processing by the non-amyloidogenic route, thereby reducing Abeta production. E2 was first demonstrated to increase secretion of the neurotrophic APPalpha while decreasing Abeta production in non-neuronal cultures (138) and has since been demonstrated in neuronal cell lines and primary neuronal cultures (139-141). The role of E2 in non-amyloidogenic APP processing is more difficult to address *in vivo*. While increased APPalpha levels have been reported in APPswe and CRND8 mice following E2 treatment (120, 121), no effect of OVX and E2 replacement was observed on APPalpha levels in guinea pigs and APP/PS1 mice, despite altered Abeta levels (118, 119). Studies in neuronal/astrocyte co-cultures indicate that astrocytes may interfere with E2 mediated regulation of APPalpha (142), providing an additional layer of complexity to the role of E2 in Abeta production.

Estrogen reduction of Abeta levels via regulation of APP processing may occur by an ER-independent mechanism. For example, ER antagonists do not block E2 mediated increases in APPalpha formation (143). Similar effects are observed in cell lines lacking functional ER (140). The pathway by which E2 may promotes non-amyloidogenic APP processing appears to involve mitogen activated protein kinase (MAPK) signaling including activation of extracellular-regulated kinases 1 & 2 (ERK1/2) (140). Similarly, testosterone has been reported to promote non-amyloidogenic APP processing through the ERK1/2 signaling pathways in cell culture models, increasing APPalpha secretion and decreasing Abeta (144, 145). However, these effects may be the result of the conversion of testosterone to E2 since pharmacological inhibition of aromatase blocks this effect (144, 145). Some evidence also suggests E2 may act through the protein kinase C (PKC) signaling pathway since pharmacological inhibition of PKC attenuates E2-mediated up-regulation of APPalpha formation (140, 146, 147).

In addition to promoting APP processing by the described non-amyloidogenic pathways, sex hormones can affect other aspects of APP metabolism that result in reduced Abeta production. For instance, E2 may actively inhibit pro-amyloidogenic APP proteolysis. Yue and colleagues reported that female APP23 mice made E2 deficient by crossing with aromatase knockout mice resulted in elevated BACE activity and corresponding increases in Abeta (76), suggesting E2 reduces Abeta by inhibiting BACE expression. This idea is reinforced by findings in the CRND8 transgenic mouse model of AD, in which E2 supplementation resulted in decreases in BACE levels, the APP fragments produced from BACE cleavage, and Abeta plaque burden (121). Recent observations in male APP23 mice crossed with aromatase knockout suggest that androgens also down regulate BACE expression and do so in a manner independent of E2 (134). Another potential mechanism by which hormones may reduce Abeta production is limiting APP substrate availability. In female animals, E2 has been shown to affect APP alternate splicing (148) and inhibit APP over-expression following ischemic injury (149). Yet, animal studies that have examined APP levels following E2 manipulation report unaltered levels (119-121). It is possible that E2 may alter APP availability for amyloidogenic metabolism without affecting total APP levels through the modulation of APP trafficking. Consistent with this possibility, E2 has been found to reduce APP trafficking to the trans-golgi network, which is the major site for amyloidogenic APP proteolysis, thereby decreasing the substrate pool for Abeta generation (150).

3.2. Sex hormones regulate beta-amyloid clearance

In addition to regulating pathways involved in Abeta production, sex hormones also reduce Abeta levels by modulating mechanisms on Abeta clearance. For example, E2 has been implicated in the clearance of Abeta through stimulation of microglial phagocytosis. In primary cultures of human microglia, E2 stimulated Abeta phagocytosis (151), while microglial cultures from E2 deficient aromatase knockout mice exhibited impaired Abeta clearance (76).

A particularly important mechanism of Abeta in clearance is the degradation of Abeta peptide monomers and oligomers by a variety of proteins collectively referred to as Abeta degrading enzymes (152). Several proteolytic enzymes in which Abeta is a suitable substrate have been identified, including neprilysin, insulin degrading enzyme, transthyretin, endothelin converting enzyme and angiotensin converting enzyme. Analysis of human control and AD cases suggests that neprilysin may be particularly important in regulating pathological accumulation of Abeta (153).

Recent findings demonstrate that both estrogens and androgens significantly increase the expression and/or activity of several Abeta degrading enzymes. In various cell culture and animal model paradigms, E2 has been

linked with the regulation of transthyretin (121, 154, 155), insulin degrading enzyme (IDE) (156), and neprilysin (157). For example, E2 increases transthyretin mRNA and protein levels in cultured epithelial cells of the choroid plexus, one of the primary sites of transthyretin synthesis (155). Further, *in vivo*, E2 administration increased transthyretin in the choroid plexus of OVX rats (155). In cultured rat hippocampal neurons, E2 was found to increase the expression of IDE, while *in vivo*, OVX was found to decrease hippocampal IDE levels, an effect that was reversed with E2 administration (156). In the same study, E2 treatment was found to promote hippocampal IDE expression while decreasing Abeta accumulation in 12 month-old 3xTgAD mice (156). In a separate study, decreased Abeta and increased cortical transthyretin and IDE levels were observed following E2 administration to CRND8 mice (121). In rats, OVX-induced E2 depletion has also been found to reduce neprilysin activity in total brain homogenate, an effect that was reversed following E2 replacement (157).

Like E2, androgens are also endogenous regulators of Abeta degrading enzymes. Although testosterone does not appear to affect expression of insulin degrading enzyme, it strongly up regulates neuronal expression of neprilysin (160). Consistent with this observation, the neprilysin gene contains at least two androgen response domains, an androgen response region (ARR) and an androgen response element (ARE) (158, 159). Androgens predominantly act through the ARE, while E2 is believed to interact via the ARR (159). Neprilysin expression and activity is modulated by androgens through a classic genomic AR-dependent mechanism, since androgen-dependent regulation of neprilysin is only observed in cultures expressing functional AR and can be inhibited with AR antagonists (160). In animals, increased Abeta and decreased neprilysin levels were observed in male rats following castration, an effect that was reversed with DHT replacement (160). Similar increases in neprilysin and associated decreases in Abeta were recently observed in male APP23 mice crossed with aromatase knockout, a genetic manipulation that increases endogenous levels of testosterone (134). Together, these findings identify sex hormones as significant regulators of Abeta degrading enzymes, a function potentially relevant AD pathogenesis and thus a promising target for therapeutic intervention (Figure 1).

4. PROGESTERONE: THE OTHER SEX HORMONE

In addition to estrogens and androgens, progesterone is increasingly considered for its potential to directly and indirectly regulate AD risk. A progestogen component is typically included in estrogen-based HT for postmenopausal women to counteract oncogenic effects of estrogens on uterus (161-164). Although less well studied in the context of AD than estrogens and androgens, progestogens may exert a range of beneficial neural actions relevant to AD (165, 166). Interestingly, both natural progesterone (P4) and synthetic progestins (e.g., medroxyprogesterone acetate) can modulate neuroprotective effects of E2, alternately negating or improving estrogen effects depending upon treatment conditions. As suggested by the cyclic nature of ovarian sex steroid hormone production, key variables in the interactions between estrogens and progestogens may include the timing and duration of hormone exposure.

In behavioral paradigms, P4 interacts with E2, often attenuating the effects of E2. Administration of E2 combined with P4 to young-adult rats was found to worsen OVX-induced impairment in the Morris water maze task, while administration of either E2 or P4 alone did not alter performance (167). In middle-aged OVX rats, progesterone reversed the beneficial effects of both tonic and cyclic E2 administration on spatial reference memory (168). In a conditioned avoidance task, E2 was found to impair performance in OVX rats, while P4 blocked E2-mediated impairment (169). Interestingly, while P4 alone did not affect conditioned avoidance performance following OVX or during diestrus (when E2 levels are low), P4 altered performance at estrus when E2 levels are elevated, suggesting that the behavioral effects of P4 were the result of interactions with E2 (169). Yet P4 does not antagonize E2-mediated cognitive benefits in all experimental paradigms, E2 combined with P4 improved spatial memory performance in aged-OVX rats equally well as E2 alone (170). Detrimental cognitive effects of combined estrogen/progestrogen HT have also been observed in humans. While estrogen alone did not affect cognition in older postmenopausal women, the combination of estrogens and a progestogen was observed to impair cognition (171).

P4 can also modulate the neuroprotective effects of E2 in experimental models of neural injury. In both young-adult and middle-aged OVX rats, P4 blocked E2-mediated protection of hippocampal neurons following kainate-induced excitotoxicity (172, 173). It is important to note that, in the absence of E2, reduced metabolites of P4 can protect against neuron loss and behavioral impairment induced by kainate (174-176). In the aged female rat, P4 blocked E2-mediated increases in neurotrophic factors including BDNF, NGF and NT3 in the entorhinal cortex (177). While either E2 or P4 alone was found to promote brain mitochondrial function in OVX rats, mitochondrial function diminished when E2 and P4 were co-administered compared to either hormone alone (178). Further, while E2 alone has been found to increase levels of the anti-apoptotic factor Bcl-2, co-administration of P4 blocks this increase (179). P4 may also attenuate some of the protective effects of E2 on AD-related neuropathology, since E2+P4 co-administration to OVX 3x-TgAD mice blocked E2-mediated reductions in Abeta accumulation (124). Despite increased Abeta deposition in E2+P4 treated mice, working memory performance was similarly improved in E2 alone and E2+P4 treated mice. Interestingly, combined E2+P4 treatment reduced tau hyperphosphorylation compared to E2 alone (124).

In some paradigms, P4 improves rather than blunts protective estrogen actions. For example, in primary cultures of hippocampal neurons, the combination of E2 and P4 potentiated neuroprotection against glutamate

toxicity compared to administration of either hormone alone (180). In female rats, P4 has been found to initially potentiate E2 mediated increases in hippocampal spine density, however, this was followed by a depletion of spine density to lower levels than those observed in untreated OVX rats (181). Other studies have found E2 combined with P4 to be equally protective as E2 alone following kainate lesion (182) and cerebral ischemia (183).

One key parameter that affects interactions between E2 and P4 is whether the hormones are delivered in a continuous or cyclic manner. For example, Gibbs *et al.* (184) found that cyclic E2+P4 administration improved cholinergic function to a greater extent than a continuous E2+P4 administration regimen. Similarly, other studies typically report benefits of P4 administered via injection, mimicking a cyclic regimen (182, 183), whereas prolonged, continuous delivery of P4 has been associated with attenuation of neuroprotective E2 effects (124, 172, 173). In female 3xTg-AD mice depleted of endogenous sex hormones by OVX, the Abeta-reducing actions of continuous E2 were blocked by continuous P4 (124, 185), but improved by cyclic P4 (185). Recently, we compared the effects of continuous versus cyclic P4 treatment regimens on neuroprotection in the entorhinal cortex following perforant path lesion, finding continuous P4 attenuated the neuroprotective effects of E2, while cyclic P4 potentiated E2-mediated neuroprotection (AMB and CJP, unpublished observations). Whether the apparent benefits of cyclic progestogen delivery suggested by recent animal studies translate to more efficacious HT in women is currently being evaluated by two ongoing clinical trials, the Early versus Late Intervention Trial with Estrogen (186) and the Kronos Early Estrogen Prevention Study (186, 187).

Although the mechanisms underlying interactions between P4 and E2 remain to be completely defined, one important area of interaction may regulation of hormone receptor expression. It is well established that levels of ERs and PRs are regulated by both E2 and P4 and that these actions can contribute to interactive hormone effects (188, 189). For example, in primary neuron cultures, P4 rapidly induced significant decreases in both ERalpha and ERbeta mRNA levels as well as reduction in ER-dependent transcriptional activity and E2 protection against apoptosis (190). Similarly, studies in cultured hippocampal slices showed that P4 blocked E2-induced increases in ERbeta expression, BDNF levels, and protection from excitotoxic challenge (191). In animal models, E2 and P4 are also associated with alterations in ERs as well as PRs, although some responses appear to be region-specific (192-197). Continued research is needed to further define molecular mechanisms underlying E2 and P4 interactions particularly as they relate to regulation of AD.

5. HORMONE THERAPY & AD

Since (1) age-related depletion of sex hormones is associated with increased AD risk, and (2) sex hormones induce specific protective actions against AD, the use of estrogen- and androgen-based hormone therapies (HT) would appear to be an obvious and effective strategy to prevent as well as treat AD. However, HT is characterized by decidedly mixed success in terms of mitigating AD risk. Although HT still retains abundant therapeutic promise, additional basic and clinical efforts are needed to realize effective use of HT as a strategy to combat AD.

While early observational and small clinical studies suggested improved cognitive abilities women with AD using estrogen-based HT (198-201), larger clinical trials later reported no cognitive benefit of HT (202-205). Although the majority of evidence suggests estrogen-based HT does not provide any benefit in the treatment of AD, the preventative potential of HT remains controversial. Numerous reports suggest that postmenopausal women treated with HT are significantly less likely to develop AD than women not receiving HT, and AD risk may be negatively associated with dose and duration of HT use (206-216). Although, some epidemiological studies report no benefit of HT on AD risk (217), these discrepancies may in part be explained by insufficient duration of HT use. For example, the Cache County Study found the greatest reduction in AD risk when HT use exceeded 10 years (212). Meta-analyses suggest that HT may reduce AD risk in the magnitude of 29-44% (218, 219). Yet the Women's Health Initiative Memory Study (WHIMS), a large randomized, double blind, placebo-controlled study reported that HT does reduce AD risk (220, 221) and may actually increase the risk of dementia (222).

To reconcile findings from a wealth of supportive findings prior clinical studies and experimental studies indicating beneficial actions of estrogens with the apparent failure of the WHIMS to confirm a protective role of HT against AD, researchers have focused their efforts on understanding the key underlying conceptual and methodological issues. Several aspects of HT have been considered, including route of HT administration (oral versus transdermal), HT regimen (continuous versus cyclic) and HT formulation (conjugated equine estrogens versus E2, interactions between E2 and progestogens) (reviewed 223). Perhaps the most significant issue is the age at which HT is initiated. An increasingly popular theory is that the onset of menopause represents a ‘window of opportunity’ during which HT must be initiated in order to realize successful neural outcomes (101, 223, 224). According to this argument, the failure of the WHIMS and select other studies to yield protection from AD is largely due to the initiation of HT many years after menopause. Consistent with this position, recent evidence demonstrated that risk of dementia in women was significantly lessened by HT use in middle age but significantly elevated by HT use in late life (225).

In contrast to the numerous studies examining the efficacy of HT in postmenopausal women, relatively few studies have evaluated testosterone-based HT for the prevention or treatment of dementia in men. Androgen therapies have been approved for the treatment of some aspects of symptomatic androgen deficiency (ADAM), including the improvement of sexual function, psychological wellbeing, muscle mass, and bone density

(226). Among the few available clinical studies of testosterone-based HT and AD, there is no data regarding the effects of HT on modifying AD risk but there is evidence that HT may provide therapeutic benefit in the management of AD. Improved spatial memory was observed in men with mild cognitive impairment and AD following six weeks of intramuscular testosterone injections (227). In a small placebo controlled clinical trial, improved quality of life and visuospatial function were observed in men with mild AD following 24 weeks of testosterone administration (228). In another small study, marked improvements in performance on the Mini Mental Status Examination and Alzheimer's Disease Assessment Scale cognitive subscale were observed in hypogonadal AD men administered testosterone compared to placebo-treated hypogonadal AD sufferers (229). While these studies are promising, large scale clinical trials need to be carried out before definitive conclusions can be drawn regarding the therapeutic or preventative potential of testosterone HT for AD. However, drawing on the experience of the outcomes of the WHIMS trials, methodological issues including delivery, formulation, administration regime, and age at initiation should be thoroughly assessed in experimental models prior to initiation of large-scale clinical trials to allow smooth translation to the clinical setting. In addition to androgen therapy for men, androgen combined with E2 therapy has also been assessed in women for the management of menopausal symptoms to improve sexual function, relieve hot flushes, improve bone density and lipoprotein profiles (reviewed 230). Whether androgen/estrogen combined HT could provide protection against AD has not been assessed, however some evidence suggests androgens are depleted in both the male and the female AD brain (27).

5.1. Hormone therapy and the aging brain

An important corollary of the 'window of opportunity' theory of HT is that protective actions of sex hormones may be muted in the aging brain. Reduced efficacy of HT in aged women is observed in several systems including bone (231) and endothelium (232). Experimental evidence in animal models supports the notion that the aging brain may also respond to the sex hormones differently than the young brain. While E2 administration decreased leakiness of the blood brain barrier in young adult OVX rats, E2 increased leakiness in reproductively senescent rats (233). In young OVX rats, long term but not short term E2 administration increases spine density in the dentate gyrus, but in aged-OVX rats, short-term but not long term E2 administration increased spine density (234). E2 administration has also been found to differentially alter the synaptic distribution of the N-methyl-D-aspartate glutamatergic receptors in the hippocampus of young compared to aged-OVX rats (235). Further, E2 treatment increases expression of the neurotrophins and neurotrophin receptors in the forebrain of young but not middle-aged rats (236).

Some behavioral effects of E2 may also be age-dependent, with improved T-maze performance observed following E2 treatment in young adult but not reproductively senescent rats challenged with the muscarinic receptor antagonist scopolamine (237). While OVX impaired spatial learning and memory performance in the Morris water maze in young-adult rats, OVX did not alter performance in middle-aged rats (238). Further, E2 replacement provided diminished benefits to water maze performance in middle aged compared to young OVX rats (238).

Although some E2 effects are diminished or altered in the aging brain, other E2 actions are conserved. For example, E2 increases choline acetyltransferase expression in both young and aged female rats (239). More recently, comparison of gene expression profiles by microarray in young and middle-aged mice revealed that E2 treatment reversed transcriptional markers of brain aging in middle-aged mice (240).

E2 also differentially modulates injury responses in young and reproductively senescent rats. For example, following perforant path deafferentation, OVX reduced hippocampal sprouting in young but not middle aged rats (241). The effects of E2 on inflammatory responses may also be modulated by age since E2 administration reduced expression of the pro-inflammatory interleukin IL-1beta following excitotoxic insult in young adult but not reproductively senescent rats (242). E2 also suppresses lipopolysaccharide-induced inflammatory cytokine expression in young adult but not reproductively senescent rats (243). Some evidence suggests that while protective in young animals, E2 may even elicit some detrimental effects in reproductively senescent animals. For example, E2 replacement decreased GFAP mRNA expression in young adult rats following perforant path transection, but increased GFAP mRNA expression in middle-aged rats (241). E2 was also found to increase severity of lesion following ischemic stroke in reproductively senescent rats, despite proving protective in young adult rats (244). In contrast, others report that E2-treated rats exhibited reduced lesion size following ischemic stroke in 9-12 month-old (245) and 16 month-old (246) female rats. However, since the acyclicity of these rats was not confirmed in the studies where neuroprotection was observed, it is possible that they may have been of heterogeneous cyclicity (245, 246).

At least some of the age-related changes in response to E2 may be the result of age-related changes in expression and/or subcellular distribution of ERs. Decreased E2 binding in nuclear extracts of middle-aged rats was the first evidence of age-related changes in ER expression and distribution (247, 248). Decreased ERalpha and ERbeta levels have since been reported in the hippocampus of aged female rats (249, 250) and the cerebral cortex of aged mice (251, 252). In aged rats, reduced expression of both ERalpha and ERbeta is observed at the pre- and post-synaptic densities (249), and up to 50% fewer spines have been found to contain ERalpha in rat hippocampus (253). Further, while the hippocampal expression of both ERalpha and ERbeta increased following E2 treatment in young adult rats, E2 up regulates hippocampal expression of ERbeta but not ERalpha in aged rats (249). In contrast, in female human

tissue, an age-related increase in ERalpha immunoreactivity has been observed in hippocampus (254).

In addition to altering sex hormone signaling mechanisms, aging also results in extended periods of hormone depletion, which in turn appear to limit efficacy of any future hormone treatment. That is, the absence of sex hormones can diminish neural responsiveness to beneficial hormone actions. In female rodents, the duration since OVX alters the efficacy of E2 treatment on hippocampal-dependent learning and memory performance (170, 255), spine density (256) and markers of cholinergic function (257). For example, improved spatial memory was observed in rats in the T-maze when E2 was administered 3 months, but not 10 months following OVX (170). Similarly, improved spatial memory performance in the radial arm maze was observed in rats administered E2 immediately but not 5 months following OVX (255). Daniel and colleagues demonstrated that E2 replacement increased hippocampal choline acetyltransferase levels when immediately administered to OVX rats, but not after a 5 month delay (257). The effects of E2 on ER expression may also change depending on the duration of hormone depletion. In middle aged rats, hippocampal ERalpha expression was increased when E2 treatment was initiated immediately following OVX increases, but not when E2 was delayed for 5 months (258).

Although less well studied, it appears that the aging male brain may also exhibit altered responsiveness to sex hormones. Most of the research on androgens and brain aging is in the area of sexual behaviors, which are positively regulated by androgen activation of androgen receptors (AR) (259). Aged male rats exhibit diminished sexual behavior that is not effectively restored by testosterone treatment (260, 261), suggesting age-related dysfunction in androgen signaling. Consistent with this possibility, in comparison to young adult male, aged male rats show low levels of nuclear AR binding that is poorly improved by testosterone treatment (262). Aged men also show evidence of similar androgen signaling disruption as indicated by age related decline of AR mRNA expression in hippocampus (263). Although the time course and underlying mechanisms of age-related changes in androgen signaling are incompletely defined, it appears that testosterone treatment is effective in middle-aged male rats in terms of regulating both AR expression and sexual behavior (259, 264). Key variables in this relationship likely include the age at which androgen treatment is initiated and the treatment duration required to retain and/or restore age-impaired androgen functions (265-267). The extent to which neuroprotective androgen signaling is altered by aging and how such changes could impact HT in aging men are significant issues that must be addressed by future research.

5.2. SERMs and SARMs: Alternatives to conventional hormone therapies

While research continues in the optimization of parameters which may determine the efficacy of estrogen-based HT for the prevention of AD in women, deleterious effects of HT including increased risk of breast cancer, cardiovascular disease and stroke (268) has lead to the investigation of the neuroprotective effects of selective estrogen receptor modulators (SERMS) as the next generation of HT (reviewed 269). SERMS elicit tissue-specific agonist and antagonistic effects. For example, the SERM raloxifene is currently used in the treatment of osteoporosis, acting as a partial estrogen agonist to prevent bone loss, while functioning as an antiestrogen in breast and endometrial tissue (270-272). In cultured neurons, low doses of raloxifene were neuroprotective against toxicity induced by Abeta, hydrogen peroxide, and glutamate (273). Raloxifene also exhibits neurotrophic effects, promoting neurite outgrowth in both PC12 cells (274) and primary neuronal cultures (273). However, raloxifene applied to neuronal cultures in combination with E2, partially inhibited the neuroprotective effects of E2 (273). In rodents, raloxifene mimicked the protective effects of E2 in a mouse model of Parkinson's disease, whereas the SERM tamoxifen partially antagonized E2 protection (275). Tamoxifen, a SERM widely used to antagonize E2 in the treatment of breast cancer, is known to block E2-mediated neuroprotection in cultures of primary neurons (276) and PC12 cells (277).

Evidence from human studies also suggests that raloxifene and tamoxifen may exhibit mixed estrogen agonist-antagonist effects in the brain. In postmenopausal women, no effect of raloxifene was observed on measures of depression, mood, and cognition following 1 year of use (278). However, in a randomized, placebo-controlled study of raloxifene administered for 3 years, the SERM was associated with a marked reduction in the risk of cognitive impairment and a mild reduction in AD risk (279). Raloxifene may increase the risk of hot flashes, suggesting an antagonist action on ER effects of vasomotor function (280, 281). Tamoxifen use has also been associated with reduced AD risk and increased independence and decision-making amongst nursing home residents (282). Further, similar profiles of markers of brain metabolism have been observed in HT and tamoxifen users compared to non-users, perhaps indicating E2 agonist effects of tamoxifen in the human brain (283). Yet, a study of breast cancer patients found increased reports of memory problems in long-term tamoxifen users (284) and impaired verbal memory (285). Because currently utilized SERMs have mixed estrogenic effects in brain, ongoing efforts have focused on the development of new SERMS that exert neuroprotective effects in the absence of oncogenic effects in reproductive tissues.

The selective ER subtype agonists propylpyrazole triol (PPT) and 2,3-bis(4-hydroxyphenol) proprionitrile (DPN), which are relatively selective for ERalpha and ERbeta respectively (286, 287), have been investigated as potential neuroprotective SERMS. Since ERbeta is expressed throughout the brain but at low levels in reproductive tissues including breast and uterus (288), compounds such as DPN may offer neuroprotective estrogenic effects in the absence of detrimental effects on reproductive tissues (reviewed 289). However, in primary hippocampal cultures, PPT but not DPN mimicked E2 and increased synaptic density (290). In primary neuronal cultures both PPT and DPN have been found to decrease

expression of the pro-apoptotic proteins and protect against glutamate (291) and Abeta-mediated cell death (292). Mixed effects of PPT and DPN have been reported in models of ischemic injury. PPT, but not DPN, was found to provide reduce cell loss in the CA1 region following ischemia in rats (293, 294). However, in mice, DPN but not PPT reduced cell loss in caudate nucleus and CA1 following global ischemia (295). In OVX 3xTg-AD mice, PPT was superior to DPN in terms of mimicking E2 effects of decreasing Abeta accumulation and improving behavioral deficits (123).

Like estrogen-based HT in women, testosterone-based HT in men is associated with potential risks. In particular, testosterone HT may have adverse effects on prostate, most notably the potential for promoting growth and/or risk of prostate tumors (5). The need for HT in men that yields androgen benefits on bone, muscle and brain but avoids deleterious consequences in prostate has driven research to develop tissue-specific selective androgen receptor modulators (SARMs). There have been several strategies in SARM development, including synthetic AR ligands that are not substrates for 5alpha-reductase and compounds that exhibit altered interaction with AR binding pocket side chains that underlie tissue specificity (296-300). Recent preclinical evidence suggests significant progress in identifying suitable candidate SARMs that exert androgenic effects on muscle at doses that do not significantly affect prostate and other reproductive tissues (300-302). Evaluation of SARMs for use neural endpoints is an essentially unexplored area, but a topic currently under investigation in the authors' laboratory.

6. PERSPECTIVE

Because AD is a disease of aging, understanding how aging promotes the disease process represents a potentially powerful approach for developing strategies to delay and perhaps prevent the disease. In this context, the normal age-related losses of sex steroid hormones in men and women appear to be significant events. In fact, abundant evidence demonstrates that low levels of sex hormones, estrogens in women and testosterone in men, are risk factors for development of AD. Basic research has identified and mechanistically characterized numerous protective actions of sex hormones that improve neural functioning and resilience and may antagonize AD pathogenesis. Not only do sex hormones increase neural plasticity and improve aspects of cognition, they also protect neurons from cell death induced by a range of toxic insults. Most importantly, sex hormones are endogenous negative regulators of Abeta, the accumulation of which initiates and drives AD cascades. Together, these lines of evidence argue that estrogen HT in women and testosterone HT men should effectively reduce AD risk and promote neural health.

Although the theory that sex hormones can protect against AD is a compelling one, clinical demonstration of HT efficacy has shown only mixed success. First, it appears that the potential benefits of estrogen- and testosterone-based HTs are largely limited to prevention rather than treatment of AD. Even in this case, emerging research indicates that there are several variables that likely impact the efficacy of HT. For example, optimal results may require that hormones be delivered transdermally rather than the traditional oral route. In addition, HT may be expected to have different effects depending upon whether it is delivered continuously or cyclically. Although sex hormone levels naturally fluctuate, testosterone rising and falling in a diurnal rhythm and E2 and P4 across the monthly ovarian cycle, the failure of HT to match the natural cyclicity of hormone levels may undermine its ability to appropriately restore normal hormone actions. Further, in the case of estrogen HT, the role of progestogens requires additional definition. New findings indicate that natural P4 and synthetic progestogens can attenuate or accentuate protective E2 actions depending upon their delivery.

Perhaps the most daunting obstacle to overcome in assessing the therapeutic potential of sex hormones is the role of aging. A key variable in the negative outcome of several HT studies appears to be the advanced age at which HT was initiated. Recent research indicates that aging male and female brains have altered, typically diminished responsiveness to sex hormones that is not ameliorated by hormone treatment during old age. Thus, efficacious HT may require initiation during middle age, a time at which sex hormone depletion is significant and yet the brain retains hormone responsiveness. However, definitive clinical evidence of that initiation of HT in middle age reduces AD risk in old age would require many years. Even in this case, important issues would need to be resolved. How long must HT be maintained in order to realize benefits, five years, ten years, more? Since prolonged HT use seems likely, adverse effects of sex hormones must be considered. Although sex hormones have numerous health benefits, they are also associated with risks including promotion of cancers in reproductive tissues. This risk may be minimized by the continued refinement of new generation SERMs and SARMs, sex hormone mimetics that exert tissue-specific agonist effects. Continuing research over the next several years should provide significant insight into these issues and determine the utility of HT for protection against AD.

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