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Androgens, andropause and neurodegeneration: exploring the link between steroidogenesis, androgens and Alzheimer's disease

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Abstract. The relationship between menopause and cognitive decline has been the subject of intense research since a number of studies have shown that hormone replacement therapy could reduce the risk of developing Alzheimer's disease in women. In contrast, research into andropause has only recently begun. Furthermore, evidence now suggests that steroidogenesis is not restricted to the gonads and adrenals, and that the brain is capable of producing its own steroid hormones, including testosterone and estrogen. Sex hormones have been demonstrated to be of critical importance in the embryonic development of the central nervous system (CNS); however, we are only just beginning to understand the role that these hormones may play in the normal functioning and repair of the adult mammalian CNS. This review will summarize current research into the role of androgens and andropause on cognition and the possible mechanisms of action of androgens, with particular reference to Alzheimer's disease.

Key words. Andropause; cognition; neurodegeneration; neurosteroids; Alzheimer's disease; testosterone.

Introduction

Female menopause is a well-established biological phenomenon characterized by a reduction in circulating levels of the female sex steroids estrogen and progesterone. This marked reduction in hormone levels has a number of physiological and psychological effects, and is linked to an increased risk of developing Alzheimer's disease (AD). Epidemiological studies indicate that women on estrogen replacement therapy (ERT) are at a reduced risk of developing AD. As such, numerous clinical trials and laboratory studies are currently being undertaken to assess the potential of estrogen as a neuroprotective agent. Research now suggests that the aging process in mal es also involves a decline in sex steroid activity. Male aging is associated with a variable but generally gradual decline in androgen activity, which can manifest as sexual dysfunction, lethargy, loss of muscle and bone mass, increased frailty, loss of balance, cognitive impairment and decreased general well being, such as depression and irritability. Andropause is defined as the partial or relative deficiency of androgens and characteristic associated symptoms. These symptoms suggest that androgens may have an important modulatory role in cognition and mental health. Indeed memory loss was the third most common reported symptom of andropause, after erectile dysfunction and general weakness in a survey of elderly men [1].

Sex steroid hormone synthesis in the CNS

Recent evidence suggests that the brain is a steroidogenic organ, with the ability to synthesie steroid hormones

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from cholesterol. The steroids produced in the brain from cholesterol and other blood-borne precursors, and that accumulate in the nervous system at a level partially independent of traditional steroidogenic organs (adrenal glands and gonads), are termed neurosteroids [2, 3]. Many neurosteroids were previously thought to have a passive role as precursors or metabolites of other steroids, however they have now been shown to have effects in the nervous system, ranging from targeting gene expression to modulating neurotransmission.

The presence of pregnenolone (PREG), dehydroepiandrosterone (DHEA) and their sulfate derivates in the brains of castrated and adrenalectomized rats provided the first evidence that the brain was capable of producing steroids [4]. Subsequent studies showed that both glial cells and neurons contain the enzymes necessary for steroid synthesis. The initial step in steroidogenesis is the conversion of cholesterol to PREG on the inner mitochondrial membrane by the enzyme cytochrome P450 side chain cleavage (P450scc). PREG can then be converted to progesterone (PROG) by the enzyme 3β-hydroxysteroid dehydrogenase-isomerase (3β-HSD) in the endoplasmic reticulum, or to DHEA by cytochrome P450c17 (P450c17, also known as 17α-hydroxylase/c17-20-lyase). The former pathway results in the synthesis of PROG and PROG metabolites such as 20α-dihydro-progesterone and 5α-dihydroprogesterone. The latter pathway culminates in the formation of testosterone via conversion of androstenedione by 17β-hydroxysteroid dehydrogenase (17β-HSD) (androstenedione is also derived from PROG). Testosterone can in turn be converted into estradiol via the enzyme P450 aromatase.

The expression of the neurosteroidogenic enzymes in the CNS is cell type specific (fig. 1). In vitro analysis of messenger RNA expression and steroid production has revealed that astrocytes are the most steroidogenic cells in the brain, expressing P450scc, P450c17, 3β-HSD, 17β-HSD and aromatase [5]. Astrocytes are therefore capable of producing PREG, PROG, DHEA, androstenedione, testosterone, estradiol and estrone. Oligodendrocytes, the myelinating cells of the CNS, express P450scc and 3β-HSD, producing PREG, PROG and androstenedione. Neurons express P450scc, P450c17, 3β-HSD and aromatase, and thus produce PREG, DHEA, androstenedione and estrogen. The relative production capability of these cells can be summarized as follows; astrocytes are the major producers of PROG, DHEA and androgens, oligodendrocytes are the predominant source of PREG and neurons are the main source of estrogens.

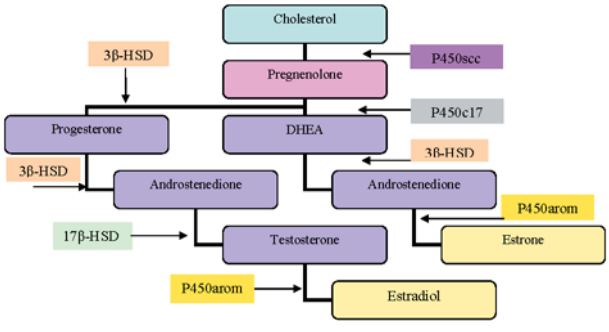

Figure 1. Sex steroid synthesis in the CNS. Major products and enzymes involved are shown. Astrocyte products are shown in purple, oligodendrocytes in pink and neurons in yellow (adapted from [3] and [5]).

Furthermore, the regulation of some neurosteroidogenic enzymes is sex specific and developmentally regulated. The expression of 3α-hydroxysteroid dehydrogenase (involved in the generation of neurosteroids through ring-A-reduction of hormonal precursors progesterone and corticosterone) is high on postnatal day 7, and is gender specific during puberty in the rat [6]. The expression of mRNA for P450scc and 11β-hydroxylase (P450c11β, involved in the synthesis of corticosterone) in the rat is region specific. P450scc mRNA is most abundant in the cortex of both male and female adult animals, and also is found in the amygdala, hippocampus and midbrain, but absent in the cerebellum and hypothalamus [7]. P450c11β mRNA is detected mainly in the amygdala and cortex, but also in the cerebellum and hippocampus of both male and female rats [7]. Interestingly, female rats have higher expression of P450c11β in the hippocampus than male rats.

Neurosteroid synthesis and metabolism is thus a complex event. Steroidogenic enzymes are differentially expressed by CNS cells, therefore adding a temporal and spatial dimension to sex steroid synthesis in the CNS. Neurosteroidogenesis can be envisaged as an autocrine event, with precursors produced by cells that are required by other cell types to produce the necessary products.

Androgens and cognition

Androgens and cognition in healthy aging

In contrast to the increasing wealth of information on the modulating effects of estrogen, the role of androgens and cognition in healthy elderly subjects is poorly understood. The significance of these few studies is further compromised by a number of experimental variables, such as age of subjects, lifestyle factors, acute or chronic illness, time of sampling, receptor status, method of measuring testosterone levels, body mass index, level of sex hormone binding globulin (SHBG), use of medication and androgen receptor polymorphism (table 1). These factors may all affect the validity of androgen assays, and thus impact upon the degree of androgen deficiency.

Testosterone

In examining the relationship between circulating levels of testosterone and cognition in older men, it is important

Table 1. Factors affecting the accuracy and interpretation of androgen assays (from Carruthers, M., ADAM: Androgen Deficiency in the Adult Male – Causes, Diagnosis and Treatment, Taylor and Francis, London, New York 2004).

The exact sampling conditions in relation to circadian and seasonal variations, diet and alcohol, physical activity and posture sample preservation and storage.
The medical problems of the patient in relation to their state of health, stress, sexual activity and smoking habits.
The accuracy and precision of the androgen analyses used which are unreliable and subject to wide inter-laboratory variation.
Androgen values are log-normally distributed, yet reference ranges are often derived from normal distribution curves, and applied to all age groups.
The levels needed later in life for optimal responses from different organs. Men may need the hormones of their youth to feel and function well, particularly if they are 'high-testosterone' men.
The interplay between testosterone, its metabolites and the combination of endogenous testosterone antagonists such as SHBG, estrogens, catecholamines, cortisol and anti-androgens.
The cellular levels of androgens needed in various organs to maintain optimal function in relation to aging endocrine systems and varying androgen receptor polymorphism.

to distinguish between total versus free levels of sex steroid hormones in plasma or serum. Total plasma testosterone represents testosterone weakly associated with albumin (54%) or bound to SHBG (44%), with the remaining 2% free to enter the brain [8]. Serum levels of bioavailable testosterone and estrogen, i.e. loosely bound or unbound to plasma proteins, were found to be associated with cognitive performance in a number of tasks in a cohort of elderly men [9]. High testosterone and low estradiol were found to improve scores on several tests of cognition. Furthermore, a U-shaped relationship was observed between sex steroid levels and certain cognitive tests, suggesting that an optimal level of sex steroids is required for some cognitive functions. Interestingly, high estradiol levels were associated with poor performance in the MMSE and BIMC (Blessed information-memory-concentration) tests.

In accordance with this study, men with higher levels of bioavailable testosterone performed better on cognitive testing (MMSE, Digit symbol and Trails B tests) compared to men with lower plasma testosterone levels [10]. This association was not found with levels of bioavailable estradiol. The levels of both estradiol and testosterone in elderly women have been found to improve verbal memory, whereas the level of sex hormones in men had no effect on verbal memory and levels of testosterone were negatively associated with verbal fluency in men [11]. Furthermore, higher free testosterone has been associated with superior performance on visual and verbal memory, visuospatial functioning and scanning, and a reduced rate of decline in visual memory [12]. The latter study did not find an association between testosterone levels and verbal ability. Spatial ability in younger men also has been linked to testosterone levels [13].

Importantly, testosterone supplementation (100–150 mg testosterone ethanate/week, intramuscularly) in elderly men has been shown to restore testosterone levels to that of younger men and improve performance in working memory tasks [14] and to improve spatial cognition [15, 16].

These divergent results for men and women underscore the idea that sex hormones modulate performance in cognitive tests in a manner similar to that expected for known sex differences. The role of sex steroid hormones in the developing nervous system is well understood in a variety of species. As such, testosterone supplementation improves performance in tasks that require spatial ability, whereas estrogen may improve verbal cognitive ability.

DHEA

Dehydroepiandrosterone (DHEA) is the most abundant steroid produced by the adrenal glands in humans. DHEA is found in circulation as the free form or bound to sulfates (DHEAS). DHEA is a precursor for estrone, estradiol and testosterone formation (fig. 1). Levels of DHEA decrease with age, illness and stress, and reduced levels of DHEA may be an important factor in the age-related vulnerability of neural tissue. A drop in DHEA levels has been speculated to have a role in many age-related diseases, including cancer, diabetes and dementia [17].

DHEA and DHEAS levels decline progressively with age such that by the age of 80, DHEA/S levels are 20% of those at age 20. The relationship between DHEA and DHEAS plasma levels and cognition in aging humans is unclear. Studies have reported cognitive dysfunction associated with low DHEAS levels, high DHEAS levels or high DHEA levels, or no relationship at all (reviewed in [18]). It would appear that health status and sex of the subject is important in the association between DHEA and cognition.

Androgen depletion and cognition

Androgen deprivation is the most common form of treatment for men diagnosed with prostate cancer for whom primary treatment has failed. Intermittent androgen suppression (IAS) involves a cycle of androgen withdrawal that lasts for 6–9 months, with a period of non-treatment to allow testosterone levels to reach physiological concentration. Once prostate-specific antigen (PSA) levels reach a threshold, IAS is readmin istered. IAS is believed to be beneficial in that it lessens the effects of androgen deprivation including those previously mentioned. A small study of men undergoing IAS via a

combination of leuprolide, a GnRH agonist, and flutamide, an androgen receptor antagonist, showed that IAS resulted in a significant reduction in spatial ability, but improved verbal ability [19]. This affect continued in the non-treatment period.

In another cohort, chemical castration by androgen depletion resulted in an increase in depression and anxiety scores [20]. Upon discontinuation of treatment, the subjects exhibited improved performance on the CAMCOG (part of the CAMDEX, a global measure of cognition and memory) and verbal recall tests.

These studies, although few in number, have clinical implications for the treatment of hormonally driven conditions, such as prostate cancer, as steroid withdrawal may result in undesirable physical and psychological side effects.

Androgens and cognition in transsexuals

The advent of sex assignment surgery has provided another human model to assess the effects of sex hormones on cognitive ability. Transsexuality is defined as incongruence between biological sex and self-declared gender identity. Male-to-female transsexuals are treated with anti-androgens in combination with estrogens, whereas female-to-male transsexuals are administered anti-androgen therapy preoperatively. This pharmacological treatment results in an improvement in visuo-spatial ability and a decrease in verbal fluency in female-to-male transsexuals [21]. Conversely, male-to-female subjects have an improved performance on verbal fluency tests, but reduced visuo-spatial ability. However, in a later study on a different group of transsexuals, testosterone was found to have a lasting effect, as male-to-female transsexuals retained their visuo-spatial ability [22]. In this second cohort, female-to-male subjects again had superior performance on visuo-spatial tasks. The authors attribute this discrepancy to the use of different cognitive tests between the two studies. An alternative explanation may be that while testosterone may play an important role in behavior and cognitive function, there may be some other underlying molecular factors behind neural sexual dimorphism. Evidence for the latter stems from a recent paper demonstrating differential gene expression between developing brains of male and female mice prior to gonadal development [23]. However, the persistent ability of these male-to-female transsexuals to exhibit enhanced visuo-spatial skills is an interesting finding that warrants further investigation.

DHEA, androgens and cognition

As discussed above, the relationship between cognition and plasma DHEA levels in humans remains controversial. However, numerous animal studies have provided insight into the possible mechanism(s) of action of DHEA in the CNS, indicating potential avenues of therapy.

DHEA and DHEAS have been shown to prevent amnesia caused by administration of dimethylsulfoxide (DMSO) in a foot shock active avoidance task in mice [24, 25]. The task assessed the memory retention capabilities of mice to avoid receiving an electrical shock using visual and audio cues in a T-maze. This effect was observed when DHEA or DHEAS was given either in the drinking water, or injected into the ventricles (intracerebroventicular injection). Intracerebroventricular administration of PREG and its metabolites (PREGS, DHEA, androstenedione, testosterone, dihydrotestosterone or aldosterone) resulted in improvement in memory retention in a foot shock active avoidance task in male mice [26]. Interestingly, estradiol and its metabolites did not have this effect. PREG and PREGS provided the most significant enhancing effect, perhaps due to the synthesis of other neurosteroids from these precursors. The memory enhancement occurred as early as 1 h post-training, suggesting the activation of immediate-early genes by these steroids.

Androgen depletion and excessive androgen levels appear to have detrimental effects on spatial learning and memory. Administration of flutamide or testosterone enanthate to the hippocampus before training increased latency times in male rats in the Morris water maze [27]. The researchers then examined the role of androgen receptors in the amygdala in Morris water maze performance. Flutamide or testosterone enanthate administration to the amygdaloid body of rats before training had no effect on escape times or distance traveled; however, testosterone administration resulted in a dose-dependent reduction in learning and memory ability [28]. The amygdala is a region rich in aromatase; thus conversion to estradiol may be the cause of this impairment. Thus, it would appear that a balance of androgen levels is required for optimal memory formation and retention.

Aged male rats treated with testosterone exhibited improved working memory and decreased nerve growth factor (NGF) levels in their hippocampi [29]. Interestingly, in this study, NGF levels were associated with poorer working memory in these aged rats. Treatment with dihydrotestosterone (DHT), which cannot be converted to estrogen by aromatase, had no effect on working memory in this model, and androgen treatment was found to lower circulating estrogen levels. These results suggest that testosterone may enhance certain memory processes independently of estradiol, as androgen treatment was found to exert a negative influence on circulating estrogen levels.

Aromatase

The presence of P450arom (aromatase) in the brain suggests that to some extent, the conversion of testosterone to estradiol is of importance to the normal functioning of the nervous system. In support of this notion

that estradiol is preferentially required by the brain, aromatase is found at higher concentrations in males rather than females, the latter presumably gaining sufficient estradiol concentrations from the periphery (i.e. ovaries) [30–32]. Aromatase activity has been shown to be neuroprotective in models of excitotoxicity in male castrated and aromatase knockout animals [33]. Low doses of domoic acid, a neurotoxic agent, resulted in neurodegeneration in the hippocampus of castrated mice. The dose administered is not neurotoxic to intact animals. Estradiol and testosterone were found to protect hippocampal neurons from domoic acid excitotoxicity. DHT treatment did not confer neuroprotection. Aromatase knockout mice also were more susceptible to excitotoxicity than their control littermates. Finally, pharmacological inhibition of aromatase with fadrozole resulted in increased neurodegeneration in male rats, an effect reversed by administration of estradiol [33]. This study suggests that the aromatization of testosterone to estradiol is a significant factor in neuroprotection afforded by sex steroids.

Androgens and glial cells

An increase in glial fibrillary acidic protein (GFAP) expression is generally observed in the aging brain [34]. Testosterone supplementation in aged rats reversed this increase in GFAP in the aged brain [35]. Astrocyte activation evidenced by increased GFAP expression has been associated with the release of inflammatory cytokines, reactive oxygen species and an alteration in the extracellular space [34, 36–40]. Thus the regulation of glial reactivity is important in the prevention of neurodegenerative diseases.

In addition to aging, astrocytes respond strongly to brain injury. A commonly employed method for simulating brain injury is to perform penetrating stab wounds to the brain. The astroglial response to such an injury is attenuated by treatment with neurosteroids, including sex steroids [41]. DHEA was found to be the most potent inhibitor of astrocyte reactivity [41].

Penetrating stab injury results in an increase in estrogen and androgen receptors in astrocytes and microglial cells, respectively [42]. Excitotoxic injury also resulted in increased glial receptor expression [42]. The morphology of astroglial cells is influenced by neurosteroids. Hippocampal astrocytes in slice cultures obtained from castrated animals had fewer GFAP positive processes compared to those from intact males [43]. Addition of sex steroids increased the number of astrocytic profiles, whereas treatment with DHEA and DHEAS induced astrocytes to form reactive profiles [43]. One of the crucial functions of astroglial cells is to maintain extracellular potassium ion concentration, and it is worth noting that the reactivity of astrocytes to high potassium levels can be modulated by neurosteroids [44].

Aromatase activity also can be induced in astroglial cells. Excitotoxic lesion induced by kainic acid and penetrating stab injury result in aromatase expression in astrocytes [45].

In summary, both neurosteroids and sex steroids are able to influence the morphology and activity of glial and neuronal cells. These effects are important in both normal and pathological CNS states.

Possible mechanisms of action on cognition by androgens

Androgens and neurotransmission

Testosterone may modulate cognitive function by influencing cholinergic neurotransmission via an increase in ACh release and by modulating nicotinic receptors [46, 47]. Neurosteroids have contrasting effects on $GABA_A$ (γ-aminobutyric acid type A) receptors. $GABA_A$ receptors are oligomeric chloride channels that, when activated, result in chloride entry into the cell, hyperpolarisation and reduced membrane excitability. Reduced metabolites of PROG and deoxycorticosterone have been shown to have an agonistic effect on $GABA_A$ receptors, resulting in an increase in chloride ion movement into the cell. In contrast, PREG-sulfate and DHEAS display $GABA_A$ antagonism, and thus induce membrane excitation [48]. The structure of these steroids, namely the presence or absence of a 3α-hydroxy group in the A-ring of the steroid, appears to be the determining factor in $GABA_A$ modulation.

Neurosteroid effects on neurotransmission are not only limited to direct receptor binding, but can be of a more complex nature. For example, estradiol metabolites and testosterone have been shown to antagonize the 5-HT_3 receptor. This activity is not mediated by binding to the receptor site (i.e. serotonin), but rather acts by insertion into the membrane at the receptor-membrane interface, thus modulating receptor activity in an allosteric manner. This relationship appears to be dependant on the structure of the steroid, the physiology of the cell membrane and the amino-acid composition of the neurotransmitter receptor itself [49].

In addition to $GABA_A$ and 5-HT_3 receptor modulation, neurosteroids have been found to interact in a structure-specific manner with NMDA (*N*-methyl-D-aspartate) and sigma receptors. DHEA, PROG and testosterone potentiate the neuronal response to NMDA in the rat hippocampus [50]. These steroids appear to act as non-selective sigma receptor antagonists, thus suppressing the activity of NMDA receptors. NMDA receptors are central to the process of excitotoxicity, as they are the receptors for the excitatory neurotransmitter glutamate [51, 52]. Gluta-

mate excitotoxicity has been implicated in Alzheimer's disease and a reduction in neurosteroid production may compromise the intrinsic defense mechanisms of the CNS to toxicity.

In summary, the interaction of neurosteroids, including androgens, with neurotransmission and neuronal excitability has a number of implications not only for cognitive disorders, but also for epilepsy, depression, alcoholism and anxiety disorders (reviewed in [49]).

Androgens, cerebrovascular disease, neuroprotection and regeneration

Cerebrovascular disease

The role of sex hormones in cardiovascular disease (CVD) is poorly understood. There is a clear sex difference in cardiovascular mortality and stroke incidence in the developed world [53], a difference that has been attributed to the possible negative vascular effects of testosterone. However, a number of more recent studies have shown that low levels of androgens are associated with increased risk of coronary heart disease, myocardial infarction and stroke [54, 55]. In addition, low testosterone was found to correlate with stroke severity and mortality, and with infarct size in elderly men [55].

The mechanism of action of androgens on the vascular system remains unclear, although a number of possibilities have been suggested. These are reviewed extensively in [56]. Testosterone has been found to have a vasodilator effect on endothelial cells via androgen receptor (AR)-dependent and -independent mechanisms [57–60]. ARs are found in all cell types of the vascular system, including endothelial cells, smooth muscle cells, cardiac myofibers, macrophages and platelets, thus providing many possible points of interaction. Testosterone also has been found to influence nitric oxide release and calcium and potassium channels in endothelial cells (reviewed in [56]). In addition, testosterone levels are inversely correlated with arterial wall stiffness in elderly men [61] and with blood pressure [54, 62]. Exogenous testosterone treatment in animal models can mediate thrombosis through enhancing platelet aggregation, thus enhancing thrombosis-, however, treatment with anabolic steroids in men have been shown to improve fibrinolytic activity and decrease serum cholesterol levels, which may promote tissue repair after ischemia [63, 64].

The relationship between androgens and CVD is further complicated by the association between testosterone levels and various CVD risk factors, including cholesterol and diabetes mellitus [65] and the possible protection afforded by aromatase conversion of testosterone to estradiol [66]. More research into androgen-mediated effects on the vascular system may yield novel preventative strategies for CVD, in particular stroke, which may further reduce the risk of neurodegeneration in later life.

Neuroprotective and anti-oxidant properties

The neuroprotective and anti-inflammatory effects of androgens and DHEA have been demonstrated in neuronal and glial cells. Androgen supplementation in culture media can induce changes in morphology, including cell size and number of processes in AR-expressing neurons [67]. Testosterone has been found to mediate neuroprotection from serum deprivation in vitro [67, 68]. Physiological concentrations of estradiol and testosterone were neuroprotective in primary cultures of human neurons under serum deprivation [68]. Non-aromatizable androgen (mibolerone) and aromatase inhibition did not block the testosterone effect. However, flutamide treatment prevented testosterone-mediated neuroprotection, thus suggesting a primary role for testosterone in neuroprotection.

DHEA and estradiol conferred a neuroprotective effect in a rat model of Parkinson's disease [69]. 1-Methyl-4-phenylpyridium (MPP+) injection is neurotoxic to the nigrostriatal dopaminergic system, resulting in a reduction in dopamine levels. DHEA and estradiol administration resulted in greater dopamine levels, retention of cholinergic fibers and reduced glial reactivity. In contrast, testosterone administration conferred no protective effect [69].

DHEAS has an immunomodulatory role on T and B lymphocytes. DHEAS also influences natural killer (NK) cell function by increasing the cytotoxic response of NK cells in healthy young and elderly subjects [70]. In Alzheimer's disease patients with low DHEAS levels, the response of NK cells is disrupted [70]. In addition, DHEA can act as an anti-oxidant and is capable of protecting hippocampal cells from the cytotoxic and lipid peroxidation effects of free radicals [71]. This effect may be due to the modulation of nuclear factor-κB (NF-κB) by DHEA. DHEA treatment resulted in a restoration of the response of diabetic rats to oxidative stress, through modulation of NF-κB activation and a restoration of the concentration of anti-oxidant defense compounds such as glutathione and catalase [72]. DHEA immunomodulatory activity also has been demonstrated in microglial cells. DHEA and DHEAS treatment prevented microglial activation by lipopolysaccharides (LPS) and beta amyloid (Aβ), resulting in a reduction in reactive nitrite production [73]. This effect appears to be post-translational in microglial cells, as mRNA levels for iNOS and the activity of NF-κB were unaffected by DHEA or DHEAS treatment.

Regeneration

DHEA and DHEAS have been shown to enhance neuronal and glial cell survival and induce differentiation

in murine embryonic cells [25]. Neurofilament protein expression was markedly increased in DHEA and DHEAS containing media. These cells had extended, thickened and intertwined processes, were in contact with neighboring neuronal cells and formed clusters. DHEA or DHEAS also improved astrocyte viability and expression of GFAP by these cells.

The beneficial role of androgens in axonal regeneration has been reviewed elsewhere [74]. Briefly, administration of testosterone immediately post-injury to castrated hamsters results in enhancement of facial motor nerve regeneration. This effect includes an increased rate of regeneration, upregulation of tubulin mRNA and ribosomal gene expression, indicating that testosterone may ameliorate the stress-like response to axotomy. Testosterone administration also reduced synaptic loss post-injury. It has been demonstrated that motor neurons contain androgen but not estrogen receptors. Coadministration of testosterone with flutamide resulted in a slowed regenerative response, compared to testosterone treatment alone in castrated animals. As such, the beneficial effect of testosterone on motor neuron regeneration appears to be via AR. The beneficial effect also was observed to a lesser extent in spinal motor neurons (as reviewed in [74]). AR receptor expression in neurons [75] and glial cells [42, 76] can be regulated by injury and circulating testosterone concentration. AR mRNA is downregulated post-orchidectomy and after axotomy [75]. AR levels also decrease with aging-especially in the cholinergic regions, the nucleus basalis of Meynert and the vertical limb of the diagonal band of Broca [77]. It has been proposed that the cholinergic activity of these areas is influenced by sex hormones in the adult human brain [9, 78]. Thus increased understanding of AR regulation and stabilization should lead to improved treatment strategies for nerve injury and trauma.

Testosterone also appears to regulate the level of nerve growth factor (NGF). Male mice have higher levels of the b subunit of NGF than females [79]. Importantly, this expression occurs in a region-specific manner. Castration resulted in a decrease in NGF levels in male mice to levels observed in intact female mice and eliminated intermale variability in NGF levels.

Thus certain neurotrophic agents and the ability of neurons to regenerate post-injury seem to be modulated by androgens. In addition, androgens appear to have important immunomodulatory functions, similar to those previously reported for estrogens.

Androgens and neurodegeneration

Androgens and Alzheimer's disease

Alzheimer's disease (AD) is a devastating neurodegenerative disorder characterized by the extracellular deposition of Aβ, a peptide derived from its parent molecule, the amyloid precursor protein (APP). Interest in the relationship of sex steroids with neurodegeneration was sparked by the observation that estrogen replacement therapy (ERT) reduced the risk of developing AD in elderly women in a number of observational case-controlled studies (reviewed in [80]). This protective effect of estrogen has been largely attributed to its ability to reduce the production of Aβ [81–84]. Subsequent studies have shown that testosterone can also regulate the production and the levels of Aβ under both in vitro [85–87] and in vivo conditions [88, 89]. The potential for androgens as therapeutic agents is currently being investigated.

As previously mentioned, IAS (intermittent androgen suppression) results in a dramatic reduction in circulating estradiol and testosterone levels. This chemical andropause is associated with a marked increase in Aβ levels in plasma [89]. More recently it has been reported that low testosterone levels are correlated with increased plasma Aβ-40 levels in elderly men with subjective memory loss or dementia [88]. In addition to this modulatory effect on Aβ production, testosterone can attenuate the toxicity of Aβ in cultured hippocampal neurons [86]. This protective effect occurred in the presence of an estrogen receptor blocker, and when non-aromatizable DHT was applied to the culture, thus suggesting that this protection is androgen mediated.

AD is also characterized by neurofibrillary tangles composed of abnormally hyperphosphorylated bundles of tau protein inside neuronal cells. Testosterone is able to prevent the heat-shock hyperphosphorylation of tau protein [90]. The heat-shock effect on tau hyperphosphorlyation is mediated in part by glycogen synthase kinase-3 beta (GSK-3 beta), the activity of which is mediated by testosterone [91]. A comparative study between age-matched controls and AD patients demonstrated a decrease in the levels of neurosteroids in many brain regions of the AD-affected brains [92]. The sulfated forms of PREG and DHEA were found at particularly low levels, in the striatum and cerebellum. In addition, the authors found a negative correlation between the levels of Aβ peptides and PREGS, and the levels of phosphorylated tau protein and DHEAS.

DHEAS plasma levels in Alzheimer patients are lower than those of the healthy aging population [70, 93, 94]. DHEA has been shown to increase the production of AβPP, leading to more AβPP in the cell membrane, and increased release of soluble AβPP fragments [95]. Thus the reduction in plasma DHEA levels in AD may lead to increased AβPP metabolism and thereby increased production of Aβ.

The epsilon 4 variant of apolipoprotein E (apoE) has been identified as a major genetic risk factor for AD [96]. Evidence suggests that this E4 effect may be more pronounced in females than males in a mouse model of

AD [97]. In female mice expressing human apoE4, androgen treatment with testosterone or DHT improved performance in spatial learning and memory tasks, with an increase in AR levels [98]. Male apoE4 mice treated with flutamide developed deficits in these tests. ApoE3 expressing male and female mice showed no effect from these treatments.

An initial small pilot study has indicated that testosterone supplementation in men with mild to moderate AD results in improvements in cognition, especially in visuo-spatial skills. Testosterone was weekly administered via intramuscular injection and resulted in an increase in bio-available and total testosterone in plasma with concomitant improvement in cognition over a 12-month period [99].

Gonadotropins and Alzheimer's disease

Luteinizing hormone (LH) also may regulate the processing of APP. Administration of leuprolide acetate, an antigonadotropin, to C57/Bl6 mice for 2 months resulted in a 3.5-fold reduction in Aβ1-42 and a 1.5-fold reduction in Aβ1-40 levels [100]. LH treatment also modulated AβPP processing to favor Aβ production in vitro [100]. Interestingly, LH has been localized to the cytoplasm of pyramidal neurons, the expression of which is higher in the AD brain compared to age-matched controls [100].

The relevance of the above findings is indicated by the elevation in serum levels of gonadotropins in male patients with AD [102]. Recently, the role of gonadotropins (LH and follicle stimulating hormone, FSH) in neurodegeneration was undertaken by our laboratory. Plasma levels of LH in a cohort of 589 post-menopausal women were found to be negatively associated with performance on the CAMCOG test [M. Rodrigues, RN Martins, unpublished observations]. In contrast, plasma FSH was positively correlated with cognition. These findings were observed in women who did not carry an APOε4 allele, and the effect was more pronounced with increasing age. FSH also was positively correlated with cognition in women who carried an apoE4 allele. These observations indicate an emerging role of gonadotropins in the processes of cognition and neurodegeneration.

Androgens and Parkinson's disease

As with AD, epidemiological studies in women have shown ERT to be associated with a reduction in risk of developing Parkinson's disease (PD) [103] and associated dementia [104]. Transdermal estradiol therapy in particular appears to show a slight pro-dopaminergic, i.e. anti-PD effect [105–107]. A high incidence of androgen deficiency has been found in male PD patients, and this, like the frequently coexisting depression, responds to testosterone treatment [108]. A Dutch study reported that a history of depression is three times as common in men with PD [109].

These results suggest that androgen depletion may play an important role in the pathogenesis of neurodegenerative diseases, such as AD and PD, by influencing the formation of the major pathological hallmarks of the disease, and by modifying genetic risk factors. Therefore, the alleviation of sex hormone deficiency in men and women may lower the risk, delay the onset and slow the progression of cognitive impairment.

Future directions

There is a clear potential for the use of steroid manipulation as a possible avenue for therapy in a number of neurodegenerative disorders, including AD and PD. The papers examined in this review point to important future directions for further investigation. As the characteristic lesions of many neurodegenerative diseases occur in a region-specific manner, it would be of interest to determine the degree of sexual dimorphism and/or region specificity of steroidogenesis in the human brain. Identification of factors that mediate steroidogenesis, and examination of the relationship between the hypothalamic-pituitary-gonadal axis and steroidogenesis, also deserves attention. In addition, further elucidation of the effects of neurosteroids on the normal function of neuronal and glial cells would yield important nowledge for the development of new therapeutic agents.

Conclusion

The intervention of modern medical techniques and improved standards of living has resulted in an aging population in many western societies. Consequently, the prevalence of aging-related diseases such as cancer, diabetes and dementia will also rise, creating a large social and economic burden. The age-related decline in sex steroids in both male and females is associated with many of these disorders, including AD. The role of these hormones in development, and in maintaining the 'status quo' in the adult CNS suggests a long-lasting effect of these hormones in neural functioning. The reported beneficial effects of steroid manipulation on cognition observed in many animal studies surely warrants further investigation into the potential of sex hormone replacement in neurodegenerative diseases. A recent report to the Alzheimer's Research Society by the London School of Economics predicts that the cost of the long-term care for elderly people with cognitive impairment in the United Kingdom is projected to more than double over the next 30 years, from £4.6 billion in 1998

to £11 billion by 2031, based upon official population projections for the increasing number of aged people [110]. Thus, there is a clear need to both develop therapeutic strategies to delay the onset and/or slow the progression of neurodegenerative diseases and to make such strategies widely available.

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