Androgens exert direct neuroprotective effects on the brain: a review of pre-clinical evidences

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Summary

Objective. To review available data describing pathways directly modulated by androgens that underlie brain neuroprotection.

Material and methods. A literature search was performed in July 2010 using the commercially available Medline online engine search to retrieve studies (from 1991 to 2010) on the mechanisms mediating the role of androgens on brain neuroprotection. A combination of the following medical subject headings was used: androgens, brain, neuroprotection, androgen receptor (AR).

Results. Direct androgen-dependent signaling pathways are active in both neuronal and glial compartments within the brain. Within physiological ranges, androgens promote β-amyloid and protein tau homeostasis, enhance antioxidant mechanisms, promote neuron viability and survival, control reactive gliosis, neuronal excitability and modulate water homeostasis.

Conclusions. Androgens directly exert neuroprotective effects in the brain thus counteracting neurodegenerative diseases and improving recovery after injury. These evidences provide rationales for androgen replacement therapy in hypogonadal subjects for the prevention, the therapy or the control of progression of neurodegenerative and neurotoxic diseases.

Key words

Androgens • Brain • Neuroprotection

Introduction

The brain is a well recognized target tissue for androgens 1. The metabolism of androgens in the brain is quite complex. Peripherally synthesized testosterone (T), mainly the bioavailable form, crosses the blood-brain barrier thanks to its lipid-permeable nature. However, brain T levels don't completely parallel circulating levels due to multiple factors: sex-hormone binding globulin, hormone transport across the blood brain barrier, and the presence of steroid converting enzymes in the brain. Moreover, the brain can directly synthesize T and other neurosteroids 2. T acts on neurons and glial cells within multiple sex-hormone sensitive brain areas through both direct androgen pathways (by itself or after 5α-reductase conversion to the nonaromatizable dihydrotestosterone (DHT)) and indirect pathways (following aromatization to estradiol or by acting on the hypothalamic-pituitary-gonadal axis) 3. T and DHT can elicit their effects via either classic genomic, or rapid non-genomic mechanisms 4. In the classic
Androgens significantly contribute to several functional and morphological aspects of brain across the lifespan. Early T exposure during prenatal and early post-natal life is involved in brain neuroplastic changes that underlie the development and the differentiation of a masculine pattern of neural and glial organization. During adolescence, T is involved in the activation of preformed brain structures leading to sexually dimorphic physical behavioral and cognitive effects. In adulthood, T exerts neuromodulatory functions that contribute to maintain brain structural and functional homeostasis.

With ageing, bioavailable plasma T levels significantly decrease in men. Using neuropathologically normal human postmortem tissues, Rosario et al. found a robust decrease in brain T levels with advancing age with minimal values in men over 80 years of age. Clinical hallmarks of brain aging include cognitive impairments such as loss of working memory, which is dependent on the prefrontal cortex and declarative (long term) memory, which is dependent on the hippocampus and other medial temporal lobe regions. Moreover, ageing is associated with the development of neurodegenerative diseases, mainly Alzheimer’s disease (AD). From a pathological point of view, brain ageing is associated with multiple degenerative processes including white matter atrophy, particularly in the frontal lobes, synaptic loss mainly in the prefrontal cortex and in medial temporal lobe structures and accumulation of beta amyloid protein (βA), the key factor involved in the pathogenesis of AD.

Does a causative nexus exist between age-associated decrease of brain T levels and the reported morpho-functional impairments? Literature suggests that androgens significantly modulate specific aspects of cognition, and that androgen depletion, either through normal aging or pharmacological action, can result in specific cognitive impairments, increased incidence of neurodegenerative diseases and worse prognosis after brain injury. Moreover, animal models show that androgen deprivation causes pathological changes that parallel those age-related discussed above, and that occur in the same brain regions. Low systemic levels of free T have been associated with impaired cognitive performances in elderly men. Men with a relatively higher free T index performed better on visual and verbal memory tasks and exhibited better long-term memory while those with low free T showed decreased visual memory, visuomotor scanning, verbal memory, and visuospatial processing. In hypogonadal men, T replacement therapy has been proved to improve some cognitive abilities, mainly verbal fluency. Free T concentrations were found to be lower in men enrolled into the Baltimore Longitudinal Study of Aging who developed AD, and this difference occurred before diagnosis. In men recently diagnosed with AD, T replacement resulted in improved performances on both the mini-mental status exam and the clock drawing test. Rosario et al. observed an approximately 50% decrease in brain T levels in men with AD aged 60-80 years in comparison to age-matched men lacking any evidence of AD or other neuropathology, an effect that was statistically significant by analysis of covariance with age as the covariable. Importantly, brain levels of T were significantly depleted in men with mild neuropathological changes to the same degree as men with severe AD neuropathology, indicating that T loss likely precedes development of AD.

Further evidences derive from patients treated with androgen deprivation therapy. Anti-androgen therapies used for the treatment of prostate cancer have been associated with cognitive impairments. Conversely, the discontinuation of anti-androgen therapy was reported to restore cognitive performances in particular verbal memory. βA levels are higher in both rodent brain and human plasma after T depriv-
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Low T levels are associated with poor outcomes after acute ischemic events in men and are inversely associated with stroke severity, infarct size, and 6-month mortality.

Why decreased brain T levels predispose to degenerative brain alterations, cognitive impairments and worse prognosis after brain injury? Recent studies demonstrate that androgens, beyond regulating sexual behavior, exert several neuroprotective functions in the brain. Neuroprotection may be defined as an effect that may result in salvage, recovery or regeneration of the nervous system, its cells, structure and function. The present review focuses on cellular and molecular mechanisms directly influenced by androgens and involved in brain neuroprotection.

Material and methods
A literature search was performed in July 2010 using the commercially available Medline online search engine. A combination of the following search terms was applied to retrieve relevant articles: androgens, brain, neuroprotection, AR. Review articles and basic studies (from 1991 to 2010) describing androgen-dependent molecular and cellular mechanisms involved in brain neuroprotection were included.

Results
Neuroprotective pathways directly regulated by androgens have been characterized in different experimental models of brain neurodegeneration and injury (Fig. 1).

Direct androgen pathways counteracting neurodegeneration

*Regulation of βA homeostasis*

βA is a proteolytic byproduct of the metabolism of the amyloid precursor protein (APP), a widely expressed transmembrane protein with numerous functions ranging from axonal transport to gene transcription. Brain levels of βA depend upon the balance between the opposing processes of βA generation and clearance. APP is metabolized by two competing pathways: the amyloidogenic and the non-amyloidogenic ones. During trafficking of APP along the non amyloidogenic pathway, proteolytic cleavage of APP by α-secretase re-
sults in secretion of a non-amyloidogenic form of soluble amyloid precursor protein (sAPPβ). The amyloidogenic pathway involves cleavage of APP by β-secretase at the amino-terminus of βA and release of sAPPβ. The remaining carboxyl-terminal fragment of APP is cleaved in the transmembrane domain by γ-secretase, generating βA peptides. Once generated, brain levels of βA are regulated by βA clearance pathways, which include the actions of the endopeptidase neprilysin (NEP) a rate-limiting βA-degrading peptidase that largely mediates steady state brain levels of βA. Brain aging and early stage AD are associated with decreased NEP levels. Androgen response elements have been identified on NEP gene affecting NEP expression. Alterations in either the production or clearance of βA that sway βA homeostasis towards increased neural levels promote intracellular and extracellular βA accumulation that abnormal assembly into oligomeric species exhibiting an altered structural conformation and capable of forming senile plaques, which are toxic for neurons, in specific brain regions. Several evidences derived from cell cultures, rodent models, and human brain demonstrate that androgens may function as endogenous regulators of βA homeostasis. Androgens regulate βA metabolism through two general pathways: direct actions through AR-dependent signals, and indirect actions through estrogen pathways (after T aromatization to estradiol) or gonadotropin pathways (via T modulation of the hypothalamic-pituitary-gonadal axis). Ramsden et al. demonstrated that DHT but not 17β-estradiol administration in adult gonadectomized Sprague-Dawley male rats reduced brain levels of soluble βA. Mechanisms by which androgens may directly affect βA levels include both regulation of βA generation and promotion of endogenous clearance pathways. McAllister C. and colleagues crossed the aromatase gene knock-out (ArKO) mice with the APP23 transgenic mice, a mouse model of AD, thus generating the APP23/Ar(±) mice in order to study the estrogen-independent effect of T on AD. Authors found, for the first time, that T administration reduced mRNA level, protein expression and activity of β-secretase in the male APP23/Ar(±) mice. Moreover, a significant increase of NEP activity was evident. Furthermore, APP23/Ar(±) mice exhibited a significant reduction in brain plaque formation and improved cognitive function when compared with age-matched male APP23 controls. Further evidence for a direct androgen action on NEP expression derives from a previous study by Yao et al. demonstrating that DHT induces a time-dependent increase in NEP expression and a decrease of βA in cultured hippocampal neurons through an AR-dependent mechanism. Zhang et al. demonstrated that physiological concentrations of androgens protect human primary neurons against intracellular βA peptide toxicity through AR by increasing the levels of heat shock protein 70 (Hsp70). The molecular mechanism underlying Hsp70 neuroprotection is not clear although multiple hypotheses have been formulated: p53 sequestration in the cytosol and prevention of its translocation to the nucleus and activation of apoptosis, direct interaction with the intracellular βA peptide, enhancement of steroid-mediated transcriptional activation of prosurvival genes.

Regulation of protein tau phosphorylation status

Protein tau is a cytosolic microtubule-associated protein that contributes to regulate the dynamism and stability of neuronal cytoskeleton by promoting microtubule assembly and stabilization. Tau metabolism is altered in several neurodegenerative diseases, as for example AD and other tauopathies. Possible mechanisms for tau alterations include abnormal phosphorylation that is responsible for the formation of aberrant tau aggregates leading to neurofibrillary degeneration. Papasozomenos and colleagues demonstrated that T but not 17β-estradiol prevents tau hyperphosphorylation in ovariectomized Sprague-Dawley rats. In addition, evidences suggest that T may also prevent calpain-mediated tau cleavage and the generation of the toxic 17-kDa tau fragment.

Direct androgen pathways involved in neuron resistance against injury

Regulation of neuron viability and survival

Ramsden et al. investigated, in an in-vivo adult male rat model, the ability of androgens to modulate neuronal loss induced by kainate, an excitotoxin that preferentially targets the hippocampus. Authors found that gonadectomy significantly increased neuronal loss induced by kainate and that DHT replacement significantly reduced the severity of neuronal loss, a finding suggesting an androgen neuroprotection exerted via an estrogen-independent pathway. Hammond et al. demonstrated that physiological T concentrations were neuroprotective on human primary brain neuron cultures by protecting them against serum deprivation mediated apoptosis. This effect was mediated by AR as it was not prevented by aromatase inhibitor but was eliminated by the anti-androgen flutamide.

Li et al. demonstrated, in a rat model, that the injection of T immediately after hypoxia-ischemia brain
damage induced by the ligation of the left carotid common artery decreased neuronal apoptosis in the cortex and hippocampus. Three main signal transduction cascades have been identified in neurons and/or glial cells that are directly modulated by androgens and that regulate cell survival: the MAPK/extracellular signal-regulated kinase (ERK) pathway (MAPK/ERK), the cAMP response element-binding (CREB) pathway and the phosphatidylinositol-3 kinase (PI3K)/Akt pathway.

MAPK/ERK cascade consists of a series of sequentially activated kinases with numerous downstream targets relevant to regulation of cell viability. Nguyen et al. found that T and DHT rapidly and transiently induced activation of MAPK/ERK in cultured hippocampal neurons as evidenced by phosphorylation of ERK-1 and ERK-2. Authors also observed that DHT was able to protect cultured astrocytes from cell death, presumably through intracellular AR and that androgens activated MAPK signaling in C6 glial cell lines, presumably through intracellular AR. Upon evaluating the effects of membrane-impermeable DHT-BSA conjugates they found an opposite effect, namely that DHT-BSA suppressed MAPK and PI3K signaling and increased cell death. Authors hypothesized that in glial cells androgens can exert opposing effects on cell signaling pathways and cell viability depending upon whether they act preferentially on membrane or intracellular receptors.

Enhancement of endogenous antioxidant mechanisms

Oxidative stress is involved in the pathogenesis of different neurological and neurodegenerative disorders such as AD, Parkinson’s Disease and Amyotrophic Lateral Sclerosis. Ahlbom et al. demonstrated, in a murine model, that cerebellar granule cells treated with T were protected from oxidative stress and cell death induced by hydrogen peroxide via an AR-mediated mechanism leading to an increase of the activity of the antioxidant enzyme catalase.

Control of reactive gliosis

Reactive gliosis is a complex phenomenon that includes a mixture of positive and negative responses critical for neuronal survival and regeneration. Reactive astrogliosis maintains the integrity of the blood-brain barrier and the survival of perilesional tissues, but may prevent axonal regeneration by forming both a mechanical and chemical barrier. Increased expression of Glial Fibrillary Acidic Protein (GFAP), an astrocyte-specific intermediate filament protein, reflects reactive astrocyte hypertrophy and is often used as an index of neurodegeneration. Reducing the amount of astrocyte reactivity during aging has been hypothesized to indirectly represent an increase in neuronal well being. Reactive microglia exerts important positive functions by remodelling the damaged tissues, but releases pro-inflammatory cytokines and may exacerbate neuronal damage. Barreto et al. evaluated the effects of early and late therapy with T or its metabolites, oestradiol and DHT, on reactive astroglia and reactive microglia after a stab wound brain injury in orchidectomized rats. Authors demonstrated that both early and delayed administration of T reduced reactive astroglia and reactive microglia and these effects may be at least in part mediated by oestradiol, while DHT may mediate part of the early effects of T on reactive mi-
Moreover, T can reverse the age-related increase in GFAP in the male rat cerebellum model. Pan et al. evaluated the role of T during recovery from neurological deficits in a rat focal ischemia model by demonstrating less GFAP expression and reactive astrocyte hypertrophy around the infarct area in T-treated rats compared with controls.

**Control of brain water homeostasis**

Aquaporin-4 (AQP4) is the most abundant water channel in the brain, where it is expressed in pericapillary astrocyte foot processes, glial limiting membranes and ependyma. AQP4-mediated transcellular water movement is crucial for fluid clearance in vasogenic brain edema following brain injury. Gu F. and colleagues demonstrated that T, but not 17β-estradiol can up-regulate AQP4 mRNA and protein expression in cultured rat astrocytes thus ameliorating their osmotic fragility from hypoosmotic stress.

**Inhibition of seizure activity**

A further mechanism of androgen protection involves attenuation of seizure severity and subsequent neuronal injury. In seizure-related lesion paradigm, Frye and colleagues found that acute androgen treatment reduced hippocampal damage. Their data suggests a protective mechanism that involves inhibition of seizure activity by the DHT metabolite 3β-diol. 3β-diol appears to attenuate seizures by modulating GABA<sub>A</sub> receptor activity to increase chloride conductance and thereby suppressing excitatory signaling.

**Conclusions**

One of the less known actions of androgens is neuroprotection. Results from literature analysis demonstrate that androgens, within physiological ranges, can favorably regulate multiple molecular pathways in the brain involved in neurodegeneration and in the response to neurotoxic stimuli through direct mechanisms. In particular, T can decrease Aβ accumulation and protein tau phosphorylation and can modulate vulnerability of neurons to toxic insults (i.e., ischemia, excitotoxicity). T-mediated neuroprotection is an intriguing field of interest from both pathophysiological and clinical point of views. The age-related depletion of T likely diminishes the ability of the brain to adequately regulate Aβ and tau protein homeostasis, resulting in increased risk for neurodegenerative diseases.

On the other hand, T replacement in aged peoples with hypogonadism may represent a valid strategy to prevent, treat, or control progression of neurodegenerative diseases and to improve neuron recovery after brain injury. Evidences described in the present review are in accordance with clinical studies demonstrating improved cognitive performances after T administration in old hypogonadal peoples and in patients with recent diagnosis of AD. However, limits from current pathophysiological knowledge should be taken in account when considering the neuroprotective therapeutic value of systemic T administration in humans. First of all, brain T levels don’t reflect exactly serum levels due to the intense metabolism the hormone undergoes (estrogen conversion by aromatase, in-loco synthesis). Moreover, most current evidences derive from pre-clinical, animal studies and may not completely reflect human brain physiology. Modern molecular neuroimaging techniques may represent a valid tool to further investigate the level of T and of its metabolites and the androgen/estrogen receptor status within specific brain regions and in different clinical settings.

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