Chapter 10

Neurophysiological Repercussions of Anabolic Steroid Abuse: A Road into Neurodegenerative Disorders

Fernando de Azevedo Cruz Seara,
Rodrigo Soares Fortunato,
Denise Pires Carvalho and
José Hamilton Matheus Nascimento

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.70475

Abstract

Since its discovery, several chemical modifications in the testosterone molecule have been done by pharmaceutical industry in order to improve its pharmacological effects, resulting in the creation of anabolic steroids (AS). Despite the therapeutic benefits, AS abuse has spread among elite and recreational athletes in the search for improvements on physical appearance and physical performance. Illicit use of anabolic AS has been correlated with several adverse effects, such as cardiovascular, endocrine, reproductive, and neurobehavioral dysfunctions. Recently, declines on cognitive and mnemonic performance have been demonstrated clinically and experimentally. Experimental studies have demonstrated that these neurological dysfunctions are correlated to spread neuronal apoptosis throughout important areas of the central nervous system (CNS), such as hippocampus and cortex. Several pathophysiological mechanisms have been linked to the AS-induced neurotoxicity, including redox imbalance and recruitment of pro-apoptotic downstream pathways. Furthermore, exposure to AS has arisen as a potential risk factor to the development of Alzheimer's disease. Altogether, these evidences imply that AS abuse *per se* induces neurodegeneration and can aggravate the prognosis of neurodegenerative diseases.

Keywords: testosterone, anabolic steroids, neurotoxicity, neurodegeneration

1. Introduction

The history of anabolic steroids (ASs) inevitably passes through the discovery of endogenous androgens. Based on previous evidences of several renowned scientists, such as Arnold Adolf

Berthold, Charles Edouard Brown-Séquard, and Fred C. Koch, the group of the pharmacologist Ernst Laqueur purified and described the chemical properties of the testicular-derived substance called testosterone. Subsequently, *de novo* synthesis of testosterone from cholesterol was described by two different groups leaded by Adolf Butendandt and Leopold Ruzicka. Thus, testosterone became not only the first hormone to be described, but also the first drug to be genuinely synthesized *in vitro*, since predecessors were plant extracts, fungi, and other sources. Conceptually, ASs are synthetic testosterone derivatives that share a common molecular structure characterized by four aromatic rings of cyclopentanoperhydrophenanthrene with 19 carbon atoms [1]. Given the structural similarities, AS can bind to androgenic receptor (AR) and exert testosterone-like physiological effects.

1.1. General pharmacological aspects of AS

Despite the molecular similarities, ASs exhibit different chemical characteristics within their structure in comparison to testosterone, which determines the differences in the pharmacological properties and physiological effects between distinct compounds. So far, three classes of AS have been described. The first class includes injectable AS with esterification of the 17β-hydroxyl group on testosterone molecule, such as testosterone propionate. This chemical modification in the structure of testosterone down-regulates the rates of absorption and degradations, resulting in substantial prolongation of the biological effects [2]. Within bloodstream, the ester bonds are rapidly hydrolyzed by blood esterases, releasing the active compound.

Like the first class, the second class of AS is composed by injectable steroids, although the biologically active compound is the 19-nor-testosterone, instead of testosterone. Furthermore, the side chain is significantly longer when compared to AS of the class I. In addition, class II ASs have a methyl group at C19 position, instead of a hydrogen atom [2]. Altogether, these chemical modifications prolong even more the rates of absorption and degradation when compared to the AS of class I. Class II includes mainly nandrolone esters, such as nandrolone decanoate and undecanoate. Basically, the longer the side chain, the prolonger the biological effect. Similarly, the esterification is rapidly hydrolyzed by blood esterases, releasing 19-nor-testosterone into the bloodstream.

The third class includes C17-alkylated AS, such as 17α-methyltestosterone, oxymetholone, methandrostenolone, and stanozolol. Given that these drugs can be orally administered, the alkylation is especially important to decrease the first-pass effect and, thus, hepatic metabolism, which could result in decreased absorption [2]. The C-1 group can also be methylated and, thus, present oral activity, but the effects induced by these drugs are relatively weaker compared to C-17-alkylated compounds.

AS compounds can be carried throughout the bloodstream by plasma proteins, such as albumin and sexual hormone-binding globulin (SHBG), or they can circulate without being conjugated. Free AS can reach target tissues and, thus, promote physiological effects. The molecular structure of AS, rich in hydrocarbons, confers to them apolar characteristics and the capacity to easily cross hydrophobic layers composed by lipids. From the systemic point of view, this characteristic allows AS to permeate physiological barriers between capillaries and

http://dx.doi.org/10.5772/intechopen.70475

target tissues, such as the blood-brain-barrier. Furthermore, from the cellular perspective, the high hydrophobicity gives AS the capacity to cross plasma membranes without necessarily binding to membrane-associated proteins.

Within target tissues, AS can undergo three different pathways. First, biological active compounds can bind directly to the target receptors, promoting their physiological effects. Second, AS can undergo 5α-reduction, by the enzyme 5α-reductase, resulting in specific metabolites [3]. This enzymatic reaction can substantially affect the physiological effects induced by the AS and must be taken into account in both therapeutic and toxicological conditions. For example, the α-reduced nandrolone-derived metabolite, 5α-dihydro-19-nor-testosterone, has a significant decreased binding affinity for the AR when compared to nandrolone, which results in decreased androgenic effects. On the other hand, dihydrotestosterone (DHT), a α-reduced testosterone-derived metabolite, has a binding affinity for the AR approximately 10-fold higher than testosterone and, thus, impose profound androgenic effects. Third, AS can be converted into estrogen by aromatase enzyme, a reaction especially observed in AS from class I [4].

Classically, AS can exert their effects by binding to AR. This receptor is a member of nuclear receptors family, which also includes estrogen, glucocorticoid, mineralocorticoid, progesterone, thyroid hormones, and retinoic acid receptors. In general, ARs have four distinct molecular domains: ligand-binding domain (LBD), which presents a canonic molecular structure among nuclear receptors; N-terminal transactivation domain, which *per se* confers the capacity of ligand-independent activity in the case of estrogen receptors; DNA-binding domain (DBD); and hinge region [5]. In the absence of agonist, the AR remains in the cytosol due to its bindings to specific chaperone proteins, such as the heat-shock-protein 90 (HSP90). These interactions are thought to be necessary for the stabilization of the receptor in an appropriate conformation that enables the steroid molecule to bind with high affinity to the LBD. Furthermore, the interaction with HSP90 prevents the AR to dimerize and bind to co-regulators.

When AS binds to the LBD, the interaction between AR and HSP90 is lost. In such a condition, active AR dimerizes, resulting in the formation of a homodimer, which is translocated into the nucleus by cytoskeleton myofilaments [6]. The interaction between the homodimer and the chromatin occurs due to the binding of zinc fingers located in the DBD at the level of androgen-responsive elements, a complex process that involves the recruitment of a cluster of co-regulators to this site [7]. Co-regulators include co-activators and co-repressors which are crucial to the transcriptional activity of the complex AR steroid. It is generally accepted that the recruitment of co-activators results in increased transcription of a target gene [8].

Besides the bioavailability within target tissues, the extent of the physiological effects induced by each AS is also correlated to the binding affinity to the AR consonant with this view; previous studies have shown that nandrolone binding affinity to the AR is 2- to 3-fold higher than testosterone-related binding affinity for the same receptor [9]. As a result, nandrolone has a more potent anabolic effect in skeletal muscle compared to testosterone [9]. Further experimental studies compared the binding affinity of nandrolone, oxymetholone, stanozolol, 17α-methyltestosterone, methenolone, methandienone, mesterolone, fluoxymesterone,

and ethyl estrenol for the AR in both skeletal muscle and prostate tissues of rodents [10]. Among these steroids, nandrolone has shown the highest binding affinity to the AR, followed by methenolone > testosterone > mesterolone. Interestingly, although the binding affinity of stanozolol, fluoxymesterone, and methandienone is thought to be significantly decreased compared with the above-mentioned steroids, the cell-based AR transactivation is comparable [11]. This evidence suggests that the degree of activation of AR does not seem to be strictly dependent on the binding affinity, despite the clear influence exerted by the latest. Indeed, gene expression can be affected in different degree by structurally distinct AS. In this context, the different conformational changes induced by distinct AS in the AR and the subsequent impact in the recruitment of co-regulators might develop a more important role in the dimension of gene expression and biological effects, although more studies are necessary to demonstrate these pharmacodynamics aspects [12].

The classical mechanism of action of AS is the AR-mediated genomic effects; however, rapid, nongenomic effects were also demonstrated for several target organs. Nongenomic effects are generally thought to request faster responses, mainly in the range of seconds to minutes, besides activation of membrane protein–mediated signaling cascades and lack of direct transcriptional/translational activation [13]. Given that rapid AS-induced responses have been observed in cell types that do not express the AR or in the presence of AR antagonists, it is reasonable to hypothesize that these effects might be triggered by mechanisms other than AR mediated. In keeping with this, experimental evidences have demonstrated that the complex AS-SHBG can bind to membrane receptors and induce increases in intracellular levels of second messengers, such as cyclic-adenosine monophosphate and inositol 1,4,5-triphosphate (IP_3), resulting in rapid cellular effects. Other studies hypothesized that AS can bind directly to noncharacterized G-protein-coupled-receptors or to nonreceptors tyrosine kinase c-SRC, which pharmacological aspects remain unclear. Furthermore, the recruitment of collateral signaling cascades by the AR activation, aside of the classical genomic mechanism, must also be taken into account [14].

Despite these divergences, evidences that AS can promote rapid changes in intracellular ion concentrations have been widely demonstrated elsewhere. Exposure of neuroblastoma cells to testosterone resulted in concentration-dependent increase of intracellular calcium in a time range of 50–100 sec [15]. Interestingly, knock down or blockade of endoplasmic reticulum IP_3 receptor ($InsP_3R$) abolished the testosterone-induced increase on intracellular calcium concentration, suggesting that $InsP_3R$-mediated testosterone effect [15]. Similarly, it has been shown that the incubation of primary hippocampal neurons with DHT-increased baseline calcium concentration [16].

Testosterone and its synthetic metabolites can modulate several physiological aspects in a wide range of cell types, and their effects can be didactically divided in androgenic and anabolic. Androgenic effects include the development of primary and secondary male sexual characteristics, the initiation and maintenance of spermatogenesis, and the maintenance of sexual behavior, such as the libido and spontaneous erections. However, as previously stated, several testosterone synthetic derivatives have lower androgenic capacity, especially those from class II.

http://dx.doi.org/10.5772/intechopen.70475

AS-induced anabolism has been observed in both experimental and clinical studies. These effects have been widely described in several target tissues and are basically related to cellular hypertrophy and hyperplasia. AS significantly potentiates nitrogen retention and protein synthesis, resulting in increased muscle mass, strength, and muscle healing, perhaps the most prominent effects aimed among bodybuilders, elite, and recreational athletes [17]. Skeletal tissue is also affected by AS, in which they stimulate osteoblasts and chondrocytes maturation, leading to epiphyseal fusion, whereas the stimulation of osteocytes promotes increases in bone formation and density. In the bone marrow, ASs stimulate the proliferation of progenitor hematopoietic cells and their maturation directly. Besides the classical androgenic/anabolic effects, AS can induce a broad spectrum of physiological effects that have been widely described and reviewed elsewhere.

1.2. Therapeutic applicability of AS

ASs were rapidly adopted as the primary therapeutic approach to treat low-circulating testosterone conditions, such as hypogonadism and andropause. Moreover, AS-related therapeutic benefits were also observed in women with endocrine dysfunctions secondary to oophorectomy and menopause. The hematopoietic effect in the bone marrow is frequently explored in the treatment of aplastic anemia and myelofibrosis. In addition, ASs are also indicated for the treatment of catabolic diseases, such as cachexia, sarcopenia, and osteoporosis correlated with malnutrition, HIV, and cancer.

ASs are considered controlled medical substances and must be used just for medical purposes and under supervision of physicians. Due to their anabolic properties, the International Olympic Committee Medical Commission banned AS from the list of substances allowed in sports competitions [18]. Currently, the rules and technical documents regarding the use of AS in sports field are under regulation of the World Anti-Doping Association. Furthermore, commercialization and consumption of AS are illegal, such as in Brazil, in the United States of America (USA), and in Great Britain.

2. Epidemiological aspects of AS abuse

Despite the beneficial therapeutic effects, illicit use of AS by individuals aiming improvements in their physical performance and esthetics has been increasingly reported during the last century. Illicit use of AS is characterized by administration of doses 10 to 1000 times higher than the doses prescribed to treat medical conditions, such as hypogonadism [19]. Dose regimens are mainly characterized by cycling, i.e., intercalation between period of time of administration and withdrawal, stacking, i.e., the combination of different types of AS, especially oral and injectable AS, and pyramiding, i.e., progressive increase of dose and frequency at a peak followed by a progressive decrease on both. Among AS users, these dose regimens are thought to supposedly reduce adverse effects associated with AS abuse, although so far, no scientific evidence supporting this hypothesis has been demonstrated.

The misuse of testosterone was firstly reported during the World War II by Nazi German army with the purpose of enhancing soldier's aggressiveness, and there has also been uncertain reports about the administration of AS in Nazi athletes during the Olympic games of 1936 [20]. During the 1940s, the use of synthetic testosterone for medical purposes spread, especially to increase sexual libido and to treat mood disorders, menorrhagia, dysmenorrhea, hypogonadism, and breast cancer [21–25]. Concomitantly, AS use was correlated with a sense of well-being and boosting of physical performance among AS users.

The reports about the relationship between the use of testosterone or its metabolites and the increase in muscle mass and strength resulted in great interest for these substances by elite athletes. It has been suggested that the first report of AS abuse by athletes was during a weightlifting championship in Vienna, 1954, by Russian weightlifters [26]. During the 1950s and 1960s, AS abuse skyrocketed among elite athletes of different countries in several categories, and obviously, it was followed by a rapid and significant increase in the athletic performance in sports competition, such as shot, hammer, and high jump [27, 28]. Interestingly, some reports suggest that popular media supported the supplementation with AS, especially with methandrostenolone, with allegations that it had no side effects [29]. Probably, the most notorious case about AS abuse by athletes involved the State Plan 14.25 of the German Democratic Republic (East German). Although AS abuse had turned into a common practice among elite athletes worldwide, East German government together with both medical and scientific communities organized massive efforts to stimulate AS administration in young and adult athletes, in order to improve their performance in Olympic games [30]. A similar sort of "governmental program" happened in the former Soviet Union between 1960s and 1970s, where it has been believed that athletes as young as 8 years were included [31]. In general, 1950s, 1960s, and 1970s decades were marked by the spread of AS administration among elite athletes. Finally, in 1974, the International Committee banned the use of testosterone and its derivatives in the Olympic games.

The first reports about AS use among bodybuilders occurred in the late 1960s and 1970s, but it was in the late 1970s and especially in the 1980s that this practice spread in this class [32]. Interestingly, this delay in comparison to elite athletes was mainly due to a current thinking that AS did not potentiate the gain of muscle mass [26]. Concomitantly, the recreational use of AS rapidly increased throughout the general population, especially in gyms. This scenario was further aggravated by the rising cult for a muscularized body shape among the general population, which was boosted by popular media [33]. Furthermore, given that this body shape paradigm has been even more complex in adolescents, AS abuse also reached high-school students [34]. In this context, underground guidelines containing information about the ways to obtain and use AS have arisen and quickly gained popularity by pseudo-scientific reports [35]. Consequently, AS abuse became a major concern to public health organizations given the severe adverse consequences that frequently follow this practice, and first, epidemiological studies have been conducted during the late 1980s and beginning 1990s showing that approximately 6.6% of 12th grade students reported AS use, and two thirds admitted its use when they were aged 16 years or less [34]. Among male Canadian adolescents, the average AS use was estimated in 5.5%, mostly stimulated by their coaches [36, 37]. In the USA,

http://dx.doi.org/10.5772/intechopen.70475

approximately one million individuals reported AS use [38]. Furthermore, AS use was positively correlated to the use of other illicit drugs, cigarettes, and alcohol [38]. Interestingly, the perception of AS abuse-induced effects among college athletes has been inversely correlated with the academic performance. In retaliation to this practice, several countries sanctioned laws prohibiting nonmedical use of AS, such as the Anabolic Steroid Act, in the USA.

Despite the classical and still frequent AS abuse among elite athletes, recent reports have suggested that the biggest group of AS users are recreational athletes and individuals aiming a supposed improvement on their esthetic appearance [39]. In the USA, epidemiological studies estimated that approximately 2.9–4.0 million Americans have used AS over their lives, a significant rise compared with data from early 1990s. Several epidemiological studies on the consumption of psychotropic drugs in Brazil have revealed that 0.9% of Brazilians have used AS, surpassing the prevalence of use of crack and heroin. Recently, it has been estimated that worldwide prevalence of AS use is approximately 3.3%, although the rate among males can reach 6.4% [40]. Middle East exhibits the highest rate of AS consumption at 21.7%, followed by South America, 4.8%; Europe, 3.8%; North America, 3.0%; Oceania, 2.6%; Africa, 2.4%; and Asia, 0.2% [40]. However, recent reports suggest that the real epidemiological extent of AS abuse might be overshadowed by the high rate of omission among AS abusers [41].

3. Adverse effects of AS abuse

Unsurprisingly, AS abuse can impose harmful adverse effects. The prevalence of these effects among AS abusers remains unclear, and recent reports have demonstrated that approximately 56% of AS users had never reported this practice to any physician, which turns the correlation between AS abuse and the adverse effects elicited by them underreported [41]. In sum, AS-induced adverse effects include reproductive, endocrinological, hepatic, cardiovascular, dermatological, and neurological dysfunctions, as demonstrated by several clinical and experimental studies. Furthermore, many effects can be persistent or even irreversible after interruption of AS use, whereas other effects arise only after AS withdrawal.

3.1. Endocrine and reproductive dysfunctions

Among adverse effects, the most common are dysfunctions in the reproductive system. Given the substantial similarity between AS and endogenous androgens, chronic use of AS results in down-regulation of both follicle-stimulating hormone and luteinizing hormone and overall suppression of the hypothalamus-pituitary-testicular (HPT) axis. Consequently, endogenous production of testosterone can be dramatically reduced, which consists in the main cause of hypogonadism in former AS users [42]. Secondary hypogonadism recovers relatively rapidly after the interruption of AS abuse, although recent reports suggest that it can last for more than a year [42]. Altogether, these abnormalities underlie the significant dysfunction in the spermatic production in AS users. When aromatizable ASs are used, secondary effects linked to increased estrogen levels can be seen, such as gynecomastia [43].

Important, but still poorly explored endocrine effects of AS abuse are those related to metabolism. In this context, abnormalities in the glucose metabolism have been reported during AS abuse, as evidenced by decreased glucose tolerance in powerlifters under AS abuse [44]. Even so, post-glucose insulin levels were increased in this condition, which suggests that AS can significantly reduce insulin sensitivity [44]. In addition, serum leptin can be significantly increased in AS abusers, without considerable changes in the adipose tissue content [45]. Interestingly, administration of nandrolone decanoate in rats can induce a significant decrease in proopiomelacortin (POMC) expression in the arcuate nucleus, despite the increased levels of leptin and insulin found in AS abusers evaluated in this study [46]. Given that anorexigenic POMC neurons can be directly activated by both insulin and leptin, these findings suggest that POMC neurons might become insensitive to these hormones, which is a common dysfunction observed in obesity and metabolic syndrome.

AS abuse has been correlated with overall down-regulation of HPT axis activity. In particular, decreased serum concentration of thyroid-stimulating hormone (TSH), thyroxine (T4), triiodothyronine (T3), free thyroxine, and thyroid-binding globulin have been found in AS abusers [47, 48]. In addition, the stimulatory effect induced by parenteral thyrotropin-release hormone (TRH) injection in the secretion of T3 can be significantly decreased by AS administration, despite the increased level of TSH observed after TRH bolus, suggesting that secondary hypothyroidism can be a prominent consequence of indiscriminate AS use [49]. On the other hand, the level of T3 can be significantly increased after AS withdrawal [47]. Experimentally, rats chronically exposed to nandrolone decanoate can also present significantly decreased serum TSH, T3, and free-T4, besides reduced hepatic deiodinase type 1 activity, followed by secondary thyroid hypertrophy [50].

3.2. Cardiovascular effects

AS abuse and cardiovascular adverse effects have long been correlated and reviewed [51]. Cardiovascular effects are marked by dyslipidemia, higher serum low-density-lipoprotein, interstitial fibrosis, cardiac hypertrophy, increased thrombogenesis, arterial hypertension, dysautonomia, and cardiac arrhythmias, as evidenced by clinical and experimental studies [52–58]. Importantly, clinical evidences suggest that some of these abnormalities, such as hypertension and dyslipidemia, are reversible after AS interruption, but others can persist for long periods or are likely irreversible. Notwithstanding in increasing the susceptibility to myocardial infarction and stroke by the above-mentioned abnormalities, chronic administration of AS has been shown to increase the damage induced by myocardial ischemia and reperfusion, which *per se* can aggravate the post-infarction prognosis [59–61]. Furthermore, recent evidences have demonstrated that therapeutic efficacy of cardioprotective maneuvers against the myocardial ischemia/reperfusion injury can be abolished by chronic exposure to AS [62]. Biochemical and molecular analyses revealed that supraphysiological doses of AS are related to redox imbalance, increased proinflammatory signaling, and overactivation of renin-angiotensin system in the heart, which seems to be closely correlated to the loss of cardioprotection after myocardial ischemia/reperfusion injury [55, 60, 61, 63].

http://dx.doi.org/10.5772/intechopen.70475

3.3. General neurological consequences

Neurological effects of AS abuse include a broad spectrum of neurobehavioral disturbances. Increased aggressiveness and violence and abnormal sexual behavior have been widely described in AS abusers, whereas anxiety and depression have been observed after AS withdrawal [64]. The behavioral abnormalities found in AS abusers seem to be correlated to profound changes in the neurochemical profile of important limbic regions, such as amygdala, hippocampal, cortical, and hypothalamic regions. These changes are probably promoted by direct bindings to the AR, which is widely expressed throughout the central nervous system, allosteric modulation of neurotransmitter receptors, or by conversion into estrogen and activation of estrogenic receptors.

Experimental studies have demonstrated that the levels of serotonin and catecholamines are closely associated with mood phenotype, motivation, anhedonia, and attention. Specifically, down-regulation of these neurotransmitters throughout limbic regions can increase the susceptibility to depression and anxiety. Interestingly, chronic exposure to AS elicited significant decrease of serotonin levels in the hippocampus, hypothalamus, cortex, and amygdala of rats [65], whereas norepinephrine and dopamine levels are up-regulated in these regions [66, 67]. In the amygdala and hypothalamus, ASs modulate the main excitatory and inhibitory neurotransmitters, namely glutamate and GABA, respectively. ASs have been shown to potentiate glutamate signaling, increasing its excitatory potential in these regions, whereas GABAergic signaling is mainly down-regulated [68, 69]. These limbic regions are associated to a broad spectrum of neurobehavioral functions that include the process of environmental information and memories, as well as the elaboration of a behavioral phenotype in response to these inputs. Therefore, the set of neurochemical alterations elicited by AS within these regions can impose remarkable neurobehavioral manifestations frequently observed in AS abusers.

Besides the neurobehavioral disturbances, AS abuse has been recently linked to loss of cognition and mnemonic performance. These evidences have been widely demonstrated in animal models of AS abuse, but so far, cognitive performance in human AS abusers remains poorly investigated. It has been reported that decline of cognition is the major consequence of the neurotoxic effect of AS, and consequently, neuronal loss in pivotal areas, such as cortex and hippocampus. Altogether, the changes in the neurophysiology elicited by supraphysiological doses of AS can substantially increase the susceptibility to neurodegenerative diseases.

4. Neurodegenerative diseases

Neurodegenerative diseases are a heterogeneous group of disorders that affect the nervous system, being primarily characterized by degeneration and dysfunction of several neural structures. Despite the efforts to develop therapeutic approaches and the significant number of studies published in this area, such findings have not resulted into development of an effective treatment so far. This lack of success is mainly attributed to the unclear

pathogenesis underlying neurodegenerative diseases, despite the well-known pathophysiological aspects, such as redox imbalance, autophagy, inflammation, and accumulation of neurotoxic substances.

In sum, it has been proposed that genetic and/or environmental factors can trigger early pathophysiological changes, such as aggregation of amyloid-β (Aβ)-protein, a common feature throughout the progression of Alzheimer's disease, resulting in primary neural damage. Subsequently, these early events can evoke secondary damages, due to inflammation, redox imbalance, and endoplasmic reticulum stress, leading to synapse dysfunctions, which has generally been accepted as a reversible process, and culminating in neuronal death and irreversible neuronal damage. Consistent evidences have implied that the accumulation of Aβ correlates—temporally and pathophysiologically—with decreased synaptic function early in the progression of Alzheimer's disease [70]. The occurrence of these events in the hippocampus has been correlated with impaired hippocampal-dependent memory consolidation. Thus, synapse dysfunction and the consequent neuronal death are the cornerstones during the progression of neurodegenerative diseases.

Conceptually, synaptic function can be modulated by distinct but correlated mechanisms. Changes in the physiological regulation of these mechanisms can lead to marked neurological effects, including cognitive impairments. First, the bioavailability of neurotransmitters is crucial to an effective synaptic function, thereby neurochemical imbalance can lead to impairment of synaptic transmission. Furthermore, post-synaptic expression of neurotransmitter receptors is equally important in the maintenance of synaptic transmission. For example, down-regulation of acetylcholine signaling in the hippocampal and cortical neuronal networks results in cognitive deficit, as observed during the progression of schizophrenia and Alzheimer's disease [71, 72]. In Parkinson's disease, loss of dopaminergic neurons located in the substantia *nigra*, and the consequent interruption of neural transmission in the nigrostriatal pathway results in a substantial drop on dopamine bioavailability in the dorsal striatum (i.e., the caudate nucleus and putamen), and decreased activity of GABAergic neurons located in the striatum [73]. Taken together, these disturbances culminate with loss of locomotor control, the most prominent characteristic of Parkinson's disease. Moreover, the pathophysiological development of neurobehavioral dysfunctions and loss of cognitive performance found in major depression are related to a decrease on the bioavailability of noradrenaline, dopamine, and 5-hydroxytryptamine [74]. Thus, drugs that enhance the bioavailability of these neurotransmitters are the first-line therapeutic approach to treat this condition.

All the above-mentioned conditions are examples of how down-regulation of synaptic transmission can induce severe degeneration in cognitive, locomotor, and behavioral control. However, synaptic function is not only negatively affected by down-regulation of neurotransmitters but also by an up-regulation of those molecules and their signaling. In some conditions characterized by increased circulating levels of glucocorticoids, such as stress and major depression, glutamatergic signaling can be significantly potentiated in hippocampal neuronal networks by cortisol and corticosterone [75]. Overactivation of glutamate receptors promote a significant increase in intracellular calcium concentration, resulting in recruitment of several downstream signaling that culminate in neuronal death, an event known as

http://dx.doi.org/10.5772/intechopen.70475

excitotoxicity. As a result, depressive patients generally have loss of hippocampal mass and mnemonic deficit. Furthermore, the extent of dendritic arborization, the density of dendritic spines, and the process of synaptogenesis are crucial aspects in the consolidation of synaptic transmission [75]. During the progression of major depression, increased levels of glucocorticoids elicit a decrease on these events in hippocampus, which contribute to the previously mentioned decline on learning and mnemonic capacities. When neurotransmitter bioavailability, receptor expression and extent of dendritic arborization are chronically up-regulated, synaptic transmission is substantially facilitated. This process is called long-term potentiation (LTP), which is thought to exert a pivotal role in the process of memory consolidation [75]. On the other hand, chronic down-regulation of these properties can elicit a process denominated long-term depression, culminating in long-term cognitive impairment.

It is important to note that all the synaptic abnormalities mentioned above can develop slowly and progressively, being frequently asymptomatic. Overtime, the spread damage culminates in functional deficit. Unfortunately, the lack of sensitive biomarkers to diagnose and to estimate the extent of these changes makes the early diagnostic of neurodegenerative diseases very difficult. As a result, this set of functional abnormalities is most commonly noted only in elderly individuals, in which the prevalence of neurodegenerative diseases is higher. Furthermore, several environmental factors can progressively increase the susceptibility to neurodegenerative diseases over lifetime, such as chronic stress and drug abuse. In this context, recent clinical and experimental findings suggest that long-term AS abuse can induce neurotoxic effects that might increase the susceptibility to loss of cognitive capacity and neurodegenerative diseases.

4.1. Pathophysiological mechanisms associated to AS-induced neurotoxicity

Studies performed in animal models and cell cultures have demonstrated a broad spectrum of pathophysiological mechanisms underlying the neurotoxicity induced by AS, and all of them seem to culminate in cell apoptosis. Apoptosis is a programmed cell death in which cell volume is progressively decreased, chromatin is condensed, and cell nucleus is fragmented [76]. Generally, apoptosis can be triggered by several distinct intracellular and extracellular stimuli, such as DNA damage, redox imbalance, calcium overload, and excitotoxicity. Naturally occurring apoptosis has been thought to exert a pivotal role in the development of multicellular organisms; moreover, it is considered a defense mechanism in several conditions, such as metabolic imbalance, infections, and neoplasia [77]. However, unbalanced apoptotic process can induce harmful effects into target tissues. In the case of cancer, for instance, the decreased apoptotic rate among neoplastic cells results in growing and spread of tumors. Conversely, increased cell death in cases of neurodegenerative diseases has been attributed to the uncontrollable apoptotic process among neuronal cells [77]. Apoptosis is triggered by two main pathways—the extrinsic, also called death receptor pathway, and the intrinsic, namely mitochondrial pathway. The extrinsic pathway is stimulated by activation of death receptors family, which includes the tumor necrosis factor (TNF)–related apoptosis-inducing ligand (TRAIL-R1 and TRAIL-R2), FAS and TNF receptors TNF)-related apoptosis-inducing ligand TNF)-related apoptosis-inducing ligand. The activation of these receptors

results in recruitment of pro-apoptotic proteins, such as BAX, BID, BAK, BAD, besides down-regulation of anti-apoptotic proteins, like Bcl-2, and culminates in activation of caspases, a family of cysteine protease enzymes, leading to cleavage of caspase substrates and cell death [77, 78]. The intrinsic apoptotic pathway is mainly triggered by intracellular stimuli, such as DNA damage, endoplasmic reticulum stress and redox imbalance. Irrespective of the central cause, these events lead to mitochondrial inner-membrane permeabilization, mainly through opening of mitochondrial permeability transition pore, culminating in mitochondrial swelling and release of apoptosis-triggering factors, such as cytochrome c [78].

Experimental studies have demonstrated that exposure to high concentrations of AS can elicit both extrinsic and intrinsic apoptosis. Long-term administration of nandrolone decanoate results in increased activation of caspase-3 and apoptosis throughout hippocampal and cortical structures [79]. Several *in vitro* studies have also demonstrated that exposure of neuroblastoma cells, primary hippocampal cells, and pheochromocytoma cells to AS can result in increased activation of caspase-3 [15, 80, 81]. Caspase-3 can be activated in both extrinsic and intrinsic apoptosis pathways and exerts a pivotal role in the execution of the apoptotic process by proteolytic cleavage of several proteins and chromatin condensation, resulting in DNA fragmentation and other changes throughout the apoptotic process. In the context of neurodegenerative diseases, caspase-3 has been shown to have a prominent role in the proteolytic cleavage of amyloid-β precursor protein and neuronal death during the progression of Alzheimer's disease [82].

Interestingly, in neuroblastoma cell culture, exposure to testosterone-induced concentration-dependent sustained increase in intracellular calcium concentration that involved up-regulation of inositol-triphosphate receptor ($InsP_3R$) type I-induced calcium release [15]. As demonstrated elsewhere, prolonged calcium overload can trigger apoptosis in several cell types [83]. Indeed, exposure to testosterone can induce caspase-3 activation in these cells, an event that can be prevented by pharmacological inhibition or knock down of $InsP_3R$ [15]. The increased activation of caspase-3 by testosterone and its synthetic metabolites induces the cleavage of poly (adenosine diphosphate-ribose) polymerase (PARP), a nuclear protein involved in DNA repair signaling [81]. In response to single-strand DNA breaks induced by cellular stressful conditions, PARP initiates the synthesis of polymeric adenosine diphosphate-ribose and leads to recruitment of DNA-repairing enzymes, such as DNA ligase and DNA polymerase [84]. As a result, cleavage of PARP by caspases can substantially increase DNA damage and apoptosis. Conversely, co-exposure with flutamide prevented the activation of caspase-3 and proteolytic cleavage of PARP, demonstrating that activation of AR is crucial to the process of DNA damage and apoptosis after exposure of neuronal cells to high concentrations of AS [81].

Furthermore, testosterone-induced activation of caspase-3 can induce proteolytic cleavage and activation of protein-kinase Cδ (PKCδ) in different cell types, including neuronal dopaminergic cell line [85]. Although the precise role of PKCδ remains controversial and experimental studies have shown both protective and pro-apoptotic effects, testosterone-induced coronary smooth muscle cell apoptosis was prevented by PKCδ and caspase-3 inhibition [86]. In addition, PKCδ has been shown to have a prominent role in the aging-related decline on hippocampal and mnemonic performance, as well as in the apoptosis of dopaminergic neurons in experimental models of Parkinson's disease [87–89]. Thus, it seems reasonable to hypothesize

http://dx.doi.org/10.5772/intechopen.70475

that PKCδ might have a pathophysiological role in AS-induced neuronal apoptosis. In contrast, the activation of ERK and Akt, two key proteins involved in the recruitment of cellular pathways linked to cell survival can be considerably decreased in neurons exposed to AS [81].

4.1.1. Redox imbalance

Redox imbalance has long been reported as a prominent mechanism underlying the apoptotic process in several pathophysiological conditions. At low concentrations, reactive oxygen species (ROS) can act as second messengers, especially hydrogen peroxide. In the thyroid gland, ROSs have been shown to have a crucial role in thyroid hormones synthesis and overall thyroid homeostasis [90]. However, in high concentration, ROS can induce oxidative damage of several cellular structures, culminating in cell death by apoptosis or necrosis [91].

Redox homeostasis is characterized by cellular antioxidant activity, such as the enzymes superoxide dismutase, catalase, thioperoxidases and glutathione complex, and ROS production by the mitochondria, nicotinamide dinucleotide phosphate oxidases (NOX), and xanthine oxidases. Thus, redox imbalance can arise in conditions of down-regulation of cellular antioxidant defense and/or ROS overproduction [90]. In the context of neurodegenerative diseases, redox imbalance has been shown to have a pivotal role in synaptic dysfunction and neuronal loss, which is observed in the brain during the development and progression of neurodegenerative diseases [92, 93]. Moreover, the hallmarks of apoptosis, including caspase activation, DNA damage, and binding of pro-apoptotic transcription factors, and cytoskeletal alterations can be strictly affected by ROS.

Redox imbalance has been reported as a prominent mechanism underlying the AS-induced cell damage and apoptosis. Experimental studies have demonstrated that chronic administration of AS can up-regulate the activity of NOX in several cell types, resulting in increased ROS production, whereas antioxidant activity seems to be substantially decreased in this condition [63, 94]. In the CNS, chronic administration of nandrolone decanoate in rats has been shown to decrease glutathione peroxidase (Gpx) activity in the hippocampus and pre-frontal cortex [79]. Gpx catalyzes the oxidation of two monomeric glutathione molecules by hydrogen peroxide into H_2O and glutathione disulfide, thus reducing the concentration of hydrogen peroxide. Thus, down-regulation of Gpx activity by AS increases hydrogen peroxide bioavailability, which is correlated to increased lipoperoxidation and reduced thiol residues induced by AS exposure in the brain [79, 95].

Interestingly, pretreatment of neuronal dopaminergic cell lines with testosterone has also been shown to protect them against oxidative damage induced by hydrogen peroxide [95]. The neuroprotective effect was correlated with a slight increase in the calcium-induced mitochondrial ROS production. In contrast, in conditions of sustained redox imbalance, post-exposure of dopaminergic neurons to testosterone can further increase the oxidative damage and decrease cell viability by mitochondrial calcium overload, an effect mediated by membrane-attached receptor [95]. Furthermore, activation of membrane-attached receptors has also been shown to be involved, as co-exposure with flutamide did prevent neither mitochondrial calcium overload nor decreased cell viability [95].

Altogether, these evidences imply that ROS might be a "switch" in the neuronal effects induced by AS, as they can determine whether exposure of neuronal cells to AS can result in neurotoxic or neuroprotective effects. In physiological concentrations, testosterone and AS can slightly increase the concentration of ROS, which might induce neuronal preconditioning, protecting these cells against further increases in ROS concentration [96, 97]. However, in supraphysiological concentrations, AS can significantly increase the ROS bioavailability by mitochondrial and nonmitochondrial mechanisms, resulting in oxidative damage and neuronal apoptosis. Interestingly, the increased susceptibility of dopaminergic neurons to AS-induced redox imbalance and oxidative damage suggests that administration of supraphysiological doses of these drugs might also increase the susceptibility to Parkinson's disease. These effects can be modulated by both membrane and cytosolic receptors, although the precise contribution of each one remains unclear. Furthermore, it remains unclear, though, whether AS exposure can induce long-term increased neuronal susceptibility to redox imbalance, as evidenced in cardiac cells [59]. Given that neurodegenerative diseases occur more frequently in elderly people, the elucidation of this aspect can develop an important role in the diagnosis and prognosis of neurodegenerative diseases in former AS abusers.

4.1.2. Excitotoxicity

The neurotoxicity induced by AS can be further complicated by the induction of excitotoxicity effect. This phenomenon occurs after a massive release of glutamate, an event called glutamate storm, or exogenous compounds, such as N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA). In such a condition, glutamate ionotropic receptors (i.e., NMDA and AMPA receptors) are overstimulated. Physiologically, activation of these receptors triggers calcium influx and plasma membrane depolarization and exerts a pivotal role in the neurotransmission and LTP process. However, overactivation of NMDA and AMPA receptors results in calcium overload, mitochondrial dysfunction, redox imbalance, and recruitment of pro-apoptotic pathways [98]. Excitotoxicity is thought to develop a prominent role in the cellular damage and tissue injury during the progression of neurodegenerative diseases, as well as major depression-associated loss of neuronal viability and decline on cognitive capacity [98].

Within physiological ranges, testosterone exhibited neuroprotective effects against neurotoxicity induced by kainic acid, an agonist of AMPA receptors and its conversion into estrogen by aromatase is thought to have a key role in this protection [99]. However, as previously stated, most of ASs are poorly converted into estrogen by aromatase, especially class II and III AS. Furthermore, the abusive characteristic of AS illicit consumption can dramatically increase the concentration of testosterone and its metabolites within neural tissue. In keeping with this, it was shown that one administration of nandrolone decanoate in rats can increase the phosphorylation of NMDA receptor subunits NR2A and NR2B in the hippocampus, suggesting that acute exposure to AS is enough to increase the activity of NMDA receptor [100]. In addition, chronic administration of AS in rats can increase the expression of vesicular glutamate transporter 2 (VGLT2) [101]. VGLT2 exerts an important role in the uptake of glutamate into synaptic vesicles, suggesting that exposure to high concentration of AS can increase not only the activity of glutamate receptors but also increase the release of glutamate into

http://dx.doi.org/10.5772/intechopen.70475

synaptic cleft. Both changes dramatically increase the susceptibility of glutamate storm and glutamate-induced excitotoxicity. Noteworthy, the increased expression of VGLT2 can persist until 3 weeks after interruption of AS administration, implying that these effects might be induced by genomic mechanisms [101].

Exposure of mixed cultures of mouse cortical cells to testosterone induced concentration-dependent increase in NMDA-induced neurotoxicity, as demonstrated by increases on trypan blue-labeling and the release of lactate-dehydrogenase [99]. This effect was further increased when aromatase inhibitors were co-administered to the culture medium, corroborating the hypothesis that testosterone-induced neuroprotection was at least partially mediated by further conversion into estrogen. In addition, co-administration of flutamide significantly attenuated the increased excitotoxicity induced by high concentrations of testosterone, highlighting the role exerted by the overactivation of AR in this regard [99]. In keeping with this, exposure of neuronal cells to nor-testosterone (i.e., nandrolone) and stanozolol increased NDMA-induced neurotoxicity in a concentration-dependent and aromatase-independent way, given that inhibition of aromatase did not attenuate this effect [99]. As a result, AS-induced potentiation in the glutamate signaling substantially increased the peak of calcium concentration induced by glutamate, whereas the return to calcium baseline levels was prolonged [16]. Conversely, inhibition of AR with flutamide completely abolished this effect. These evidences imply that ASs potentiate excitotoxicity induced by overactivation of glutamate receptor exclusively via classic AR pathway.

Besides the potentiation of glutamate-induced excitotoxicity, experimental studies have shown that exposure of neuronal cells to AS can also modulate the neurotoxic effects of Aβ. These oligomers are physiologically generated by cleavage of amyloid precursor protein (APP) by β- and γ-secretases, and the β-site APP-cleaving enzyme 1 (BACE1) is the most prominent β-secretase throughout the brain. The most common isoforms are $A\beta_{40}$ and $A\beta_{42}$, where the shorter (i.e., $A\beta_{40)}$ is produced in the trans-Golgi apparatus and is the most prominent, whereas the longer is produced in the endoplasmic reticulum and has the most notorious fibrillogenic capacity. The clearance of Aβ is performed by several pathways, including activation of degrading enzymes and receptor-mediated cellular and vascular clearance.

Under unclear circumstances, though, Aβ generation and clearance can be unbalanced, resulting in accumulation and aggregation of Aβ [102]. In this context, presenilin 1 (PS1) and presenilin 2 (PS2) regulate the proteolytic function of γ-secretases, and recent studies have demonstrated that mutations in both protein can result in accumulation of $A\beta_{42}$, which is the hallmark of Alzheimer's disease [103]. Aggregated Aβ can induce neurotoxic effects by several mechanisms, including induction of calcium overload and redox imbalance, culminating in synaptic deterioration and neuronal apoptosis [102]. Furthermore, soluble Aβ, also known to induce neurotoxicity, is increased in the cerebrospinal fluid of patients with Alzheimer's disease [104, 105]. Noteworthy, Aβ can bind to NMDA and AMPA receptors, and these interactions can further increase excitotoxicity induced by glutamate [106].

Recently, it has been demonstrated that Aβ levels can be substantially increased in the whole brain and cerebrospinal fluid, but especially in the hippocampus, after short-term exposure to 17β-trenbolone in male rats [80]. Similarly, exposure of primary hippocampal neurons

to 17β-trenbolone, but not to DHT, can significantly elevate the levels of $A\beta_{42}$. Interestingly, this effect was seen only in male rats. Even so, administration of 17β-trenbolone in pregnant female rats resulted in accumulation of $A\beta_{42}$ in the fetus brain [80]. In keeping with these findings, exposure to 17β-trenbolone also resulted in a concentration-dependent down-regulation of PS1 levels in primary hippocampal neurons [80]. These evidences have demonstrated that AS abuse can induce accumulation of $A\beta_{42}$ in the hippocampus, which can induce long-term susceptibility to Alzheimer's disease, especially in the offspring of female AS abusers.

Notwithstanding the effects with respect to the synthesis of Aβ, AS can modulate the toxicity induced by these oligomers. In this context, testosterone *per se* can induce neuroprotective effect against the toxicity induced by Aβ, an effect observed in mixed cortical neuronal cell cultures [107]. This effect was completely abolished by co-exposure with aromatase, suggesting that aromatization and the local generation of estrogen can underlie the testosterone-induced neuroprotection. Interestingly, co-exposure with the AR antagonist flutamide can also attenuate the neuroprotection induced by testosterone against Aβ-induced toxicity, implying that activation of classical AR can attenuate the toxicity induced by Aβ oligomers [107]. In addition, the exposure of neuronal cells to nandrolone, a poorly aromatizable AS, did not affect the neurotoxicity induced by Aβ, whereas it was significantly potentiated by exposure to methandrostenolone [107]. However, nandrolone-BSA (bovine serum albumin) conjugate significantly potentiated the Aβ-induced neurotoxicity, whereas the conjugation of BSA further increased the neurotoxic potentiation induced by methandrostenolone *per se*.

Taken together, these evidences have demonstrated that AS not only increases the generation of Aβ oligomers in crucial areas of CNS associated with the cognitive and mnemonic capacities but also increases the neurotoxic effect of these molecules, which can substantially increase the susceptibility to Alzheimer's disease. The mechanism underlying the elevated level of Aβ remains unclear but might involve disturbances in the organelles, where Aβ is produced (i.e., endoplasmic reticulum and Golgi apparatus); moreover, the role of endoplasmic reticulum stress must be investigated in this regard. Furthermore, the downstream signaling underlying the AS-induced potentiation in the neurotoxic effect promoted by Aβ seems to involve membrane receptors instead of the classical cytosolic AR. Thus, this effect might be more pronounced in drugs that exhibit increased binding affinity for the membrane receptor and that are poorly converted into estrogen by aromatase.

4.2. Long-term AS abuse and cognitive impairments

Testosterone and other endogenous androgens *per se* have been shown to exert a pivotal role during the development of CNS. In keeping with this, recent evidences have demonstrated that the development of central nervous system exhibits sexual dimorphic differences, including the size of cortical and sub-cortical structures. In addition, cognitive and mnemonic performances can be strikingly influenced by the levels of testosterone and estrogen within CNS. Indeed, decreased levels of testosterone have been correlated to a poor performance in cognitive tests, increased levels of Aβ throughout the brain, and increased susceptibility to Alzheimer's disease [108]. Conversely, testosterone replacement can significantly restore

http://dx.doi.org/10.5772/intechopen.70475

these abnormalities [109, 110]. Despite these evidences regarding endogenous testosterone levels, consistent data have shown that long-term administration of supraphysiological doses of AS can significantly impair the cognitive capacity. Evaluation of cognitive capacity involves mainly standard tests including attention and psychomotor tests, functional executive tests, memory tests, and emotional/social cognition tests.

In recent studies, long-term AS users submitted to cognitive analysis have performed significantly worse in visuospatial memory tests and learning capacity compared to nonusers [111, 112]. Basically, AS users made more mistakes in the attempt to recognize visual patterns they had seen immediately before the test. The capacity to distinguish between new and already seen visual pattern was also impaired in AS users when compared to nonusers. In addition, AS users showed a tendency to make more mistakes in the attempt to memorize verbal patterns 30 minutes after the presentation. Interestingly, cognitive impairments were significantly correlated to the total lifetime of AS dose consumption [112]. In another recent study, AS users had worse results in tests evaluating attention and inhibitory control skills [111]. Taken together, these findings evidence that AS abuse can elicit substantial cognitive loss and raise the question of whether the effects of long-term consumption can be even more remarkable with aging. Noteworthy, adolescents have exhibited more sensitivity to AS-induced cognitive impairments when compared to adult AS users, which suggest that chronic AS abuse during pubertal and pre-pubertal phases might induce more severe neurological impairments [111]. Importantly, these individuals might be more susceptible to aging-associated loss of cognitive capacity when compared to individuals that started AS abuse in adulthood.

Studies focused in animal models of AS abuse have shown conflicting findings, depending on the test and dose regimen used. In the passive avoidance test, rodents undergo fear-motivated analyses of short-term and long-term memory, as they learn to avoid their innate tendency for preference dark environments, instead of bright areas, by exposure to aversive stimulus (i.e., electric shock) in the dark area. Long-term administration of nandrolone decanoate (4 mg/week) for 10 weeks significantly increased the extinction of learned responses (i.e., avoidance of the learned aversive stimulus) when compared to vehicle-treated rats, suggesting that nandrolone-impaired mnemonic performance, whereas rats administered with testosterone enanthate performed better than control rats [113]. In contrast, one injection of nandrolone decanoate (1–6 mg/rat) or testosterone enanthate (5–30 mg/rat) significantly improved the mnemonic performance of rats in the passive avoidance test [114]. These evidences suggest that long-term exposure to poorly aromatizable AS, such as nandrolone, can considerably impair learning and memory consolidation, whereas treatment with aromatizable AS might improve these aspects, probably by increasing local production of estrogen in the CNS.

In the Morris water maze test, visuospatial memory is evaluated by repeated presentations of rats to the maze in daily training trials, in which rats must find the target platform. In the day of the test, the latency time spent to find the central platform, the time spent within the target platform, and the time spent in the surrounding areas of the maze are compared between experimental groups. In this context, administration of AS cocktail (2 mg/kg testosterone cypionate, 2 mg/kg nandrolone decanoate, and 1 mg/kg boldenone undecylenate) or 0.375 mg/kg

methandrostenolone for 10 weeks did not affect the performance, as the latency time to find the target platform was statistically equivalent to the level of vehicle-treated rats. In contrast, after 4 training trials, rats chronically treated with nandrolone decanoate (15 mg/kg each third day, for 14 days) exhibited increased latency time to reach the target platform, whereas the time spent within the target platform was significantly decreased when compared to the control group [115]. Interestingly, long-term AS administration *per se* not only impaired learning and mnemonic performances but also abrogated the well-known improvements in these skills elicited by chronic treadmill exercise. In sum, treadmill exercised rats chronically administered with nandrolone decanoate spent significantly more time to find the target platform in the Morris water maze when compared to exercised control rats, whereas the time spent in the target platform was significantly decreased [116].

Besides the impairment of spatial memory, exposure to nandrolone decanoate also reduces the social memory capacity of rats [117]. In social memory capacity evaluation, adult rats are allowed to investigate and recognize juvenile rats for 5 minutes. During this period of time, adult rats frequently demonstrate investigatory-like behavior, such as head and body sniffing, anogenital exploration, grooming, close pursuing, touching the flanks with the snout, and manipulation with the forepaws. After an interval of time, the same juvenile rats are reintroduced to the adult rat. In this context, long-term administration of nandrolone decanoate (15 mg/kg, daily, for 6 weeks) significantly increased the recognition time in the second exposure, when compared to control rats [117]. Importantly, this effect was completely abolished when flutamide, an AR antagonist, was co-administered with nandrolone. These findings suggest that long-term exposure to nandrolone impaired the mnemonic capacity by stimulation of AR within CNS.

Taken together, these evidences corroborate the findings in AS abusers that the loss on mnemonic capacity might be proportional to the dose and time of AS exposure, as well as the compound administered (i.e. aromatizable or nonaromatizable). More studies are necessary to elucidate whether longer exposure to AS and more cycles can further impair cognitive capacity in experimental models. Furthermore, the impact of chronic administration of AS in aging rats should be investigated, given that the majority of oldest AS abusers in general population (i.e., that started to use AS in the 1970s and 1980s) are entering the age of risk of ND now [112].

Despite the evidences about decline of cognitive and mnemonic capacities after AS administration, the underlying mechanisms are complex and remain unclear so far. Experimental studies have shown that high concentrations of AS can elicit apoptosis of several cell types, including cardiomyocytes, endothelial, and skeletal muscles cells. Even so, the overall consequences of AS exposure on neural cells viability remain poorly explored in *in vivo* studies. *In vitro* studies have demonstrated decreased cell viability in neural cell cultures exposed to AS, suggesting that neuronal loss might be the central event in the cognitive decline during supraphysiological AS intake. These neuronal adverse effects are especially critical in the case of AS abuse, given the capacity of these drugs to cross the blood-brain barrier and accumulate in the neural tissue. In keeping with this, short-term administration of 17β-trenbolone, a class

http://dx.doi.org/10.5772/intechopen.70475

II AS, in adult males and females, and pregnant female rats, resulted in accumulation of AS throughout the brain and cerebrospinal fluid, but especially in the hippocampus [80].

Hippocampus is a sub-cortical region that develops a pivotal role in the consolidation of new memories and spatial cognition. Bilateral destruction of hippocampus impairs the formation of new episodic memories and induces anterograde and retrograde amnesia in epileptic patients [118]. Hippocampus has also been correlated to the consolidation of episodic and declarative memories through the process of LTP [119]. Interestingly, experimental studies have demonstrated that specific neuronal clusters within the hippocampus are activated when rats and monkeys pass through particular locations, which suggest that there is a "neuronal mapping" associated with distinct environments [119]. Noteworthy, studies have demonstrated that in several conditions characterized by cognition and memory decline, such as Alzheimer's disease and other forms of dementia, the hippocampus is one of the earliest structures to exhibit synaptic dysfunctions [120].

The density of AR in the hippocampus is the highest of CNS; thus, it is particularly sensitive to oscillation in circulating levels of testosterone [121, 122]. Exposure of neuroblastoma cell culture to different testosterone concentrations induced a concentration-dependent decrease on cell viability [15]. This effect was also observed in primary culture of hippocampal neurons, in which the incubation for 48 hours with 17β-trenbolone significantly decreased cell viability [80]. Furthermore, administration of nandrolone decanoate (15 mg/kg, daily) for 5 days in adult males, females, and pregnant female rat (embryonic day 15) resulted in a significant decrease of BrdU-labeled cells in the dentate gyrus of the hippocampus, indicating that AS overdose decreased cell proliferation [123].

The dentate gyrus is a hippocampal area at the interface of entorhinal cortex and CA3 region of hippocampus [124]. Excitatory inputs from the layer II of the entorhinal cortex project to the dentate gyrus, which send neuronal projections to the CA3 region via mossy fibers. This trisynaptic circuit exerts a particular role in the process of spatial memory and cognition. In keeping with this, experimental studies have demonstrated that neuronal death in the dentate gyrus granule cells resulted in significantly decreased performance on hippocampal-sensitive memory tests, such as the Morris water maze, acquisition of reference, and working memory tests [125, 126]. Worth of noting long-term administration of nandrolone decanoate (10 mg/kg/week, for 8 weeks) in rats significantly decreased neuronal density not only in the dentate gyrus but also throughout CA1, CA2, CA3, pre-frontal cortex, and parietal cortex [79].

Noteworthy, the dentate gyrus is one of the few regions of the adult brain to exhibit neurogenesis and acute nandrolone administration decreased the number of newly born neurons within dentate gyrus of adult rats in approximately 75%, implying that short-term administration of AS is enough to significantly impair neurogenic processes [123]. Furthermore, neuronal loss and impaired neurogenesis in the hippocampal and cortical structures have been correlated to the development of Alzheimer's disease-related cognitive decline, as well as to increased number of Aβ plaques in this region [127]. Conversely, experimental evidences have demonstrated that both aerobic and anaerobic exercises can significantly increase neuronal

proliferation in these regions, an effect that has been correlated to the improved mnemonic capacity and neuronal survival [128, 129]. In this context, rats submitted to strength exercise in a vertical ladder showed up-regulation of Ki-67 throughout the dentate gyrus, which is considered a marker of neurogenesis [130]. However, chronic administration of nandrolone decanoate abolished this effect, suggesting that AS abuse can decrease the neurogenic effect induced by exercise.

In cortical neuronal and astrocytic cultures, 48 hours of exposure to 10 mM of testosterone, nandrolone, or methandrostenolone significantly increased neuronal death [107]. Interestingly, when these ASs were conjugated to BSA, which impedes the AS to cross the plasma membrane and to bind to the cytosolic AR, the neurotoxicity was further increased. Even so, co-exposure of flutamide prevented both testosterone- and nandrolone-induced neurotoxicity, suggesting that the membrane-attached AR shares pharmacological similarities with the cytosolic receptor [107]. Furthermore, these evidences suggest that activation of membrane-attached AR can recruit distinct downstream signaling pathways that culminate in enhanced cell death, when compared to the cytosolic AR.

Taken together, these clinical and experimental evidences imply that chronic exposure to supraphysiological doses of AS can severely impair cognitive and mnemonic capacities. This paradigm might be worsened by aging-related neurophysiological effects, which can increase the susceptibility to neurodegenerative diseases, besides the well-described neurobehavioral effects.

5. Conclusions

The growing misuse of AS is a major concern worldwide due to its harmful effects, including cardiovascular, endocrine, reproductive, behavioral, and neurological abnormalities. Unfortunately, there are several unclear aspects regarding the consequences of AS abuse, such as the prevalence of adverse effects, the repercussions in aging-related dysfunctions, such as neurodegenerative diseases, and if these effects are reversible. Even so, experimental studies have provided consistent evidences that the short-term and long-term exposure to AS can induce neuronal apoptosis throughout important neural regions, such as hippocampus and pre-frontal cortex. As a result, this phenomenon can severely impair cognitive and mnemonic capacities, as evidenced by clinical studies with AS abuses. In addition, exposure to AS can significantly increase the susceptibility to Alzheimer's disease. Taken together, these evidences support the hypothesis that administration of supraphysiological doses of AS is an important risk factor to the development of neurodegenerative diseases, and that the prognosis of these conditions might be worsened by AS abuse. Given the rising misuse of AS among elite athletes and recreational users, these neurological consequences should not be underestimated by physicians and researchers. The understanding of these aspects is particularly important to provide the diagnostic and prognostic of neurological diseases in active and former AS abusers.

Acknowledgements

This study was supported by the Brazilian Council for Scientific and Technological Development (CNPq), the Rio de Janeiro State Research Foundation (FAPERJ) and the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES). JHMN, DPC and RSF are research fellows from the CNPq and FACS receive a fellowship from the CNPq.

Author details

Fernando de Azevedo Cruz Seara[1], Rodrigo Soares Fortunato[2], Denise Pires Carvalho[3] and José Hamilton Matheus Nascimento[1]*

*Address all correspondence to: jhmn@biof.ufrj.br

1 Laboratory of Cardiac Electrophysiology, Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

2 Laboratory of Molecular Radiobiology, Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

3 Laboratory of Endocrine Physiology, Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

References

[1] World Health organization. Guidelines for the Use of Androgens in Men. Geneva: WHO; 1992

[2] Basaria S, Wahlstrom JT, Dobs AS. Anabolic-androgenic steroid therapy in the treatment of chronic diseases. Journal of Clinical Endocrinology and Metabolism. 2001;**86**:5108-5117. DOI: 10.1210/jcem.86.11.7983

[3] Grino PB, Isidro-Gutierrez RF, Griffin JE, Wilson JD. Androgen resistance associated with a qualitative abnormality of the androgen receptor and responsive to high dose androgen therapy. Journal of Clinical Endocrinology and Metabolism. 1989;**68**:578-584. DOI: 10.1210/jcem-68-3-578

[4] Simpson ER, Davis SR. Minireview: Aromatase and the regulation of estrogen biosynthesis – some new perspectives. Endocrinology. 2001;**142**:4589-4594. DOI: 10.1210/endo.142.11.8547

[5] Shaffer PL, Jivan A, Dollins DE, Claessens F, Gewirth DT. Structural basis of androgen receptor binding to selective androgen response elements. Proceedings of National

Academy of Sciences United States of America. 2004;**101**:4758-4763. DOI: 10.1073/pnas.0401123101

[6] Kuil CW, Berrevoets CA, Mulder E. Ligand-induced conformational alterations of the androgen receptor analyzed by limited trypsinization: Studies on the mechanism of antiandrogen action. Journal of Biological Chemistry. 1995;**270**:27569-27576. DOI: 10.1074/jbc.270.46.27569

[7] Schoenmakers E, Alen P, Verrijdt G, Peeters B, Verhoeven G, Rombauts W, Claessens F. Differential DNA binding by the androgen and glucocorticoid receptors involves the second Zn-finger and a C-terminal extension of the DNA-binding domains. Biochemical Journal. 1999;**341**:515-521. DOI: 10.1042/bj3410515

[8] Perissi V, Rosenfeld MG. Controlling nuclear receptors: The circular logic of cofactor cycles. Nature Reviews Molecular Cell Biology. 2005;**6**:542-554. DOI: 10.1038/nrm1680

[9] Sundaram K, Kumar N, Monder C, Bardin CW. Different patterns of metabolism determine the relative anabolic activity of 19-norandrogens. Journal of Steroid Biochemistry and Molecular Biology. 1995;**53**:253-257. DOI: 10.1016/0960-0760(95)00056-6

[10] Saartok T, Dahlberg E, Gustafsson JA. Relative binding affinity of anabolic-androgenic steroids: Comparison of the binding to the androgen receptors in skeletal muscle and in prostate, as well as to sex hormone-binding globulin. Endocrinology. 1984;**114**:2100-2106. DOI: 10.1210/endo-114-6-2100

[11] Feldkoren BI, Andersson S. Anabolic-androgenic sterowid interaction with rat androgen receptor in vivo and in vitro: A comparative study. Journal of Steroid Biochemistry and Molecular Biology. 2005;**94**:481-487. DOI: 10.1016/j.jsbmb.2004.12.036

[12] Holterhus PM, Piefke S, Hiort O. Anabolic steroids, testosterone-precursors and virilizing androgens induce distinct activation profiles of androgen responsive promoter constructs. Journal of Steroid Biochemistry and Molecular Biology. 2002;**82**:269-275. DOI: 10.1016/S0960-0760(02)00220-0

[13] Foradori CD, Weiser MJ, Handa RJ. Non-genomic actions of androgens. Frontiers in Neuroendocrinology. 2008;**29**:169-181. DOI: 10.1016/j.yfrne.2007.10.005

[14] Losel RM, Falkenstein E, Feuring M, Schultz A, Tillmann HC, Rossol-Haseroth K, Wehling M. Nongenomic steroid action: Controversies, questions, and answers. Physiological Reviews. 2003;**83**:965-1016. DOI: 10.1152/physrev.00003.2003

[15] Estrada M, Varshney A, Ehrlich BE. Elevated testosterone induces apoptosis in neuronal cells. Journal of Biological Chemistry. 2006;**281**:25492-25501. DOI: 10.1074/jbc.M603193200

[16] Foradori CD, Werner SB, Sandau US, Clapp TR, Handa RJ. Activation of the androgen receptor alters the intracellular calcium response to glutamate in primary hippocampal neurons and modulates sarco/endoplasmic reticulum calcium ATPase 2 transcription. Neuroscience. 2007;**149**:155-164. DOI: 10.1016/j.neuroscience.2007.06.054

[17] Bhasin S, Storer T, Berman N, Callegari C, Clevenger B, Phillips J, et al. The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. New England Journal of Medicine. 1996;**335**:1-7. DOI: 10.1056/NEJM199607043350101

[18] Sjöqvist F, Garle M, Rane A. Use of doping agents, particularly anabolic steroids, in sports and society. Lancet. 2008;**371**:1872-1882. DOI: 10.1016/S0140-6736(08)60801-6

[19] Cohen J, Collins R, Darkes J, Gwartney D. A league of their own: Demographics, motivations and patterns of use of 1,955 male adult non-medical anabolic steroid users in the United States. Journal of the International Society of Sports Nutrition. 2007;**4**:1-14. DOI: 10.1186/1550-2783-4-12

[20] Beamish R, Ritchie I. The spectre of steroids: Nazi propaganda, cold war anxiety and patriarchal paternalism. International Journal of the History of Sport. 2005;**22**:777-795. DOI: 10.1080/09523360500143406

[21] Altschule MD, Tillotson KJ. The use of testosterone in the treatment of depressions. New England Journal of Medicine. 1948;**239**:1036-1038. DOI: 10.1056/NEJM194812302392704

[22] Adair FE, Herrmann JB. The use of testosterone propionate in the treatment of advanced carcinoma of the breast. Annals of Surgery. 1946;**123**:1023-1035

[23] Hellers C, Myers G. The male climacteric, its symptomatology, diagnosis and treatment: Use of urinary gonadotropins, therapeutic test with testosterone propionate and testicular biopsies in delineating the male climacteric from psychoneurosis and psychogenic impotence. Journal of the American Medical Association. 1944;**126**:472-477. DOI: 10.1001/jama.1944.02850430006003

[24] Hurxthal LM. Sublingual use of testosterone in 7 cases of hypogonadism: Report of 3 congenital eunuchoids occurring in one family. Journal of Clinical Endocrinology and Metabolism. 1943;**3**:551-556. DOI: 10.1210/jcem-3-10-551

[25] Salmon UJ, Geist SH. Effect of androgens upon libido in women. Journal of Clinical Endocrinology. 1943;**30**:235-238. DOI: 10.1210/jcem-3-4-235

[26] Wade N. Anabolic steroids: Doctors denounce them, but athletes aren't listening. Science. 1972;**176**:1399-1403. DOI: 10.1126/science.176.4042.1399

[27] Payne AH. Anabolic steroids in athletics (or the rise of the mediocrity). British Journal of Sports Medicine. 1975;**9**:83-88. DOI: 10.1136/bjsm.9.2.83

[28] Jones CS. Massive overdose of adrenaline. British Medical Journal. 1967;**4**:683-684

[29] Freed D, Banks AJ, Longson D. Anabolic steroids in athletics. British Medical Journal. 1972;**3**:761

[30] Dickman S. East Germany: Science in the disservice of the state. Science. 1991;**254**:26-27. DOI: 10.1126/science.1925557

[31] Riordan J. The rise and fall of soviet olympic champions. Olympika: The International Journal of Olympic Studies. 1993;**2**:25-44

[32] Friedl KE, Yesalis CE. Self-treatment of gynecomastia in bodybuilders who use anabolic steroids. Physician and Sports Medicine. 1989;**17**:67-79. DOI: 10.1080/00913847.1989.11709731

[33] Featherstone M. The body in consumer culture. Theory, Culture and Society. 1982;**1**:18-33. DOI: 10.1177/026327648200100203

[34] Buckley WE, Yesalis CE, Friedl KE, Anderson WA, Streit AL, Wright JE. Estimated prevalence of anabolic steroid use among male high school seniors. Journal of the American Medical Association. 1988;**260**:3441-3445. DOI: 10.1001/jama.1988.03410230059028

[35] Duchaine D. Underground Steroid Handbook II. Venice: HLR Tech Books; 1989. p. 95

[36] Newman S. Despite warnings, lure of steroids too strong for some young canadians. Canadian Medical Association Journal. 1994;**151**:844-846

[37] Canadian Centre of Drug-free Sport. National School Survey on Drugs and Sport: Final Report. Ottawa: Canadian Centre for Ethics in Sports; 1993. 41p

[38] Yesalis CE, Kennedy NJ, Kopstein AN, Bahrke MS. Anabolic-androgenic steroid use in the United States. Journal of the American Medical Association. 1993;**270**:1217-1221. DOI: 10.1001/jama.1993.03510100067034

[39] Baker J, Thomas N, Davies B. Anabolic androgenic steroid (AAS) abuse: Not only an elite performance issue. Open Sports Medicine Journal. 2008;**2**:38-39. DOI: 10.2174/1874387000802010038

[40] Sagoe D, Molde H, Andreassen C, Torsheim T, Pallesen S. The global epidemiology of anabolic-androgenic steroid use: A meta-analysis and meta-regression analysis. Annals of Epidemiology. 2014;**24**:383-398. DOI: 10.1016/j.annepidem.2014.01.009

[41] Pope HG, Kanayama G, Ionescu-Pioggia M, Hudson JI. Anabolic steroid users' attitudes towards physicians. Addiction. 2004;**99**:1189-1194. DOI: 10.1111/j.1360-0443.2004.00781.x

[42] Rahnema CD, Lipshultz LI, Crosnoe LE, Kovac JR, Kim ED. Anabolic steroid-induced hypogonadism: Diagnosis and treatment. Fertility and Sterility. 2014;**101**:1271-1279. DOI: 10.1016/j.fertnstert.2014.02.02

[43] Calzada L, Torres-Calleja J, Martinez JM, Pedrón N. Measurement of androgen and estrogen receptors in breast tissue from subjects with anabolic steroid-dependent gynecomastia. Life Sciences. 2001;**69**:1465-1469. DOI: 10.1016/S0000004-3205(01)0122227-9

[44] Cohen JC, Hickman R. Insulin resistance and diminished glucose tolerance in powerlifters ingesting anabolic steroids. Journal of Clinical Endocrinology and Metabolism. 1987;**64**:960-963. DOI: 10.1210/jcem-64-5-960

[45] Hislop MS, Ratanjee BD, Soule SG, Marais AD. Effects of anabolic-androgenic steroid use or gonadal testosterone suppression on serum leptin concentration in men. European Journal of Endocrinology. 1999;**141**:40-46. DOI: 10.1530/eje.0.1410040

[46] Lindblom J, Kindlundh AM, Nyberg F, Bergström L, Wikberg JE. Anabolic androgenic steroid nandrolone decanoate reduces hypothalamic proopiomelanocortin mRNA levels. Brain Research. 2003;**986**:139-147. DOI: 10.1016/S0006-8993(03)03223-2

[47] Daly RC, Su TP, Schmidt PJ, Pagliaro M, Pickar D, Rubinow DR. Neuroendocrine and behavioral effects of high-dose anabolic steroid administration in male normal volunteers. Psychoneuroendocrinology. 2003;**28**:317-331. DOI: 10.1016/S0306-4530(02)00025-2

[48] Alèn M, Rahkila P, Reinilä M, Vihko R. Androgenic-anabolic steroid effects on serum thyroid, pituitary and steroid hormones in athletes. American Journal of Sports and Medicine. 1987;**15**:357-361. DOI: 10.1177/036354658701500411

[49] Deyssig R, Weissel M. Ingestion of androgenic-anabolic steroids induces mild thyroidal impairment in male body builders. Journal of Clinical Endocrinology and Metabolism. 1993;**76**:1069-1071. DOI: 10.1210/jcem.76.4.8473383

[50] Fortunato RS, Marassi MP, Chaves EA, Nascimento JHM, Rosenthal D, Carvalho DP. Chronic administration of anabolic androgenic steroid alters murine thyroid function. Medicine and Science in Sports and Exercise. 2006;**38**:256-261. DOI: 10.1249/01.mss.0000183357.19743.51

[51] Maior AS, Belchior C, Sanches RC, Oliveira T, Leonelli T, Schwingel PA, et al. Chronic users of supraphysiological doses of anabolic androgenic steroids develop hematological and serum lipoprotein profiles that are characteristic of high cardiovascular risk. International Journal of Sport and Exercise Science. 2011;**3**:27-36

[52] Maior AS, Carvalho ARA, Marques-Neto SR, Menezes P, Soares PP, Nascimento JHM. Cardiac autonomic dysfunction in anabolic steroid users. Scandinavian Journal of Medicine and Science in Sports. 2013;**23**:548-555. DOI: 10.1111/j.1600-0838.2011.01436.x

[53] Maior AS, Menezes P, Pedrosa RC, Carvalho DP, Soares PP, Nascimento JH. Abnormal cardiac repolarization in anabolic androgenic steroid users carrying out submaximal exercise testing. Clinical and Experimental Pharmacology and Physiology. 2010;**37**:1129-1133. DOI: 10.1111/j.1440-1681.2010.05452.x

[54] Maior AS, Simão R, Salles BF, Alexander JL, Rhea M, Nascimento JH. Acute cardiovascular response in anabolic androgenic steroid users performing maximal treadmill exercise testing. Journal of Strength and Conditioning Research. 2010;**24**:1688-1695. DOI: 10.1519/JSC.0b013e3181 dc46c9

[55] Marques Neto SR, Silva AH, Santos MC, Ferraz EF, Nascimento JHM. The blockade of angiotensin AT_1 and aldosterone receptors protects rats from synthetic androgen-induced cardiac autonomic dysfunction. Acta Physiologica. 2013;**208**:166-171. DOI: 10.1111/apha. 12056

[56] Medei E, Marocolo M, Rodrigues DC, Arantes PC, Takiya CM, Silva J, Rondinelli E, Goldenberg RC, Carvalho AC, Nascimento JH. Chronic treatment with anabolic steroids induces ventricular repolarization disturbances: Cellular, ionic and molecular mechanism. Journal of Molecular and Cellular Cardiology. 2010;**49**:165-175. DOI: 10.1016/y.jmcc.2010.04.014

[57] Olivares EL, Silveira AL, Fonseca FV, Silva-Almeida C, Côrtes RS, Pereira-Junior PP, Nascimento JH, Reis LC. Administration of an anabolic steroid during the adolescent phase changes the behavior, cardiac autonomic balance and fluid intake in male adult rats. Physiology and Behaviour. 2014;**126**:15-24. DOI: 10.1016/j.physbeh.2013.12.006

[58] Pereira-Junior PP, Chaves EA, Costa-e-Sousa RH, Masuda MO, de Carvalho ACC, Nascimento JHM. Cardiac autonomic dysfunction in rats chronically treated with anabolic steroid. European Journal of Applied Physiology. 2006;**96**:487-494. DOI: 10.1007/s00421-005-0111-7

[59] Seara FAC, Barbosa RAQ, Oliveira DF, Gran da Silva DL, Carvalho AB, Ferreira ACF, Nascimento JHM, Olivares EL. Administration of anabolic steroid during adolescence induces long-term cardiac hypertrophy and increases susceptibility to ischemia/reperfusion injury in adult Wistar rats. J Steroid Biochem Mol Biol. 2017;**171**:34-42. DOI:10.1016/j.jsbmb.2017. 01.012

[60] Marques-Neto SR, Ferraz EB, Rodrigues DC, Njaine B, Rondinelli E, Campos de Carvalho AC, Nascimento JH. AT1 and aldosterone receptors blockade prevents the chronic effect of nandrolone on the exercise-induced cardioprotection in perfused rat heart subjected to ischemia and reperfusion. Cardiovasc Drugs Therapy. 2014;**28**:125-135. DOI: 10.1007/s10557-013-6503-8

[61] Chaves EA, Pereira-Junior PP, Fortunato RS, Masuda MO, Carvalho AC, Carvalho DP, Oliveira MF, Nascimento JH. Nandrolone decanoate impairs exercise-induced cardioprotection: Role of antioxidant enzymes. Journal of Steroid Biochemistry and Molecular Biol. 2006;**99**:223-230. DOI: 10.1016/j.jsbmb.2006.01.004

[62] Penna C, Tullio F, Perrelli MG, Moro F, Abbadessa G, Piccione F, Carriero V, Racca S, Pagliaro P. Ischemia/reperfusion injury is increased and cardioprotection by a postconditioning protocol is lost as cardiac hypertrophy develops in nandrolone treated rats. Basic Research in Cardiology. 2011;**106**:409-420. DOI: 10.1007/s00395-010-0143-y

[63] Chaves EA, Fortunato RS, Carvalho DP, Nascimento JHM, Oliveira MF. Exercise-induced cardioprotection is impaired by anabolic steroid treatment through a redox-dependent mechanism. Journal of Steroid Biochemistry and Molecular Biology. 2013;**138**:267-272. DOI: 10.1016/jsbmb.2013.06.006

[64] Clark AS, Henderson LP. Behavioral and physiological responses to anabolic-androgenic steroids. Neuroscience and Biobehavioral Reviews. 2003;**27**:413-436. DOI: 10.1016/S0149-7634(03)00064-2

[65] Kubala KH, McGinnis MY, Anderson GM, Lumia AR. The effects of an anabolic androgenic steroid and low serotonin on social and non-social behaviors in male rats. Brain Research. 2008;**1232**:21-29. DOI: 10.1016/j.brainres.2008.07.065

[66] Frahm KA, Lumia AR, Fernandez E, Strong R, Roberts JL, McGinnis MY. Effects of anabolic androgenic steroids and social subjugation on behavior and neurochemistry in male rats. Pharmacology Biochemistry and Behavior. 2011;**97**:416-422. DOI: 10.1016/j.pbb.2010.09.015

[67] Ricci L, Schwartzer J, Melloni R. Alterations in the anterior hypothalamic dopamine system in aggressive adolescent AAS-treated hamsters. Hormones and Behavior. 2009;**55**:348-355. DOI: 10.1016/j.yhbeh.2008.10.011

[68] Carrillo M, Ricci LA, Melloni RH. Developmental and withdrawal effects of adolescent AAS exposure on the glutamatergic system in hamsters. Behavioral Neurosciences. 2011;**125**:452-464. DOI: 10.1037/a0023475

[69] McIntyre KL, Porter DM, Henderson LP. Anabolic androgenic steroids induce age-, sex-, and dose-dependent changes in $GABA_A$ receptor subunit mRNAs in the mouse forebrain. Neuropharmacology. 2002;**43**:634-645. DOI: 10.1016/S0028-3908(02)00154-5

[70] Villemagne VL, Burnham S, Bourgeat P, Brown B, Ellis KA, Salvado O, et al. Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: A prospective cohort study. Lancet Neurology. 2013;**12**:357-367. DOI: 10.1016/S1474-4422(13)70044-9

[71] Wood S, Cummings JL, Hsu MA, Barclay T, Wheatley MV, Yarema KT, Schnelle JF. The use of the neuropsychiatric inventory in nursing home residents. Characterization and measurement. American Journal of Geriatric Psychiatry. 2000;**8**:75-83

[72] Koukouli F, Rooy M, Tziotis D, Sailor KA, O'Neill HC, Levenga J, et al. Nicotine reverses hypofrontality in animal models of addiction and schizophrenia. Nature Medicine. 2017;**23**:347-354. DOI: 10.1038/nm.4274

[73] Alonso Cánovas A, Luquin Piudo R, García Ruiz-Espiga P, Burguera JA, Campos Arillo V, Castro A, Linazasoro G, López Del Val J, Vela L, Martínez Castrillo JC. Dopaminergic agonists in Parkinson's disease. Neurologia. 2014;**29**:230-241. DOI: 10.1016/nrl.2011.04. 012

[74] Heninger GR, Delgado PL, Charney DS. The revised monoamine theory of depression: A modulatory role for monoamines, based on new findings from monoamine depletion experiments in humans. Pharmacopsychiatry. 1996;**29**:2-11. DOI: 10.1055/s-2007-979535

[75] Duman RS, Malberg J, Thome J. Neural plasticity to stress and antidepressant treatment. Biological Psychiatry. 1999;**46**:1181-1191. DOI: 10.1016/S0006-3223(99)00177-8

[76] Renehan AG, Booth C, Potten CS. What is apoptosis, and why is it important? British Medical Journal. 2001 Jun;**322**:1536-1538. DOI: 10.1136/bmj.322.7301.1536

[77] Putcha GV, Harris CA, Moulder KL, Easton RM, Thompson CB, Johnson EM. Intrinsic and extrinsic pathway signaling during neuronal apoptosis: Lessons from the analysis of mutant mice. Journal of Cell Biology. 2002;**157**:441-453. DOI: 10.1083/jcb.200110108

[78] Sinha K, Das J, Pal PB, Sil PC. Oxidative stress: The mitochondria-dependent and mitochondria-independent pathways of apoptosis. Archives of Toxicology. 2013;**87**:1157-1180. DOI: 10.1007/s00204-013-1034-4

[79] Kumral A, Ozer E, Yilmaz O, Akhisaroglu M, Gokmen N, Duman N, Ulukus C, Genc S, Ozkan H. Neuroprotective effect of erythropoietin on hypoxic-ischemic brain injury in neonatal rats. Biology of the Neonate. 2003;**83**:224-228. DOI: 10.1016/j.neulet.2012.10.004

[80] Ma F, Liu D. 17β-trenbolone, an anabolic–androgenic steroid as well as an environmental hormone, contributes to neurodegeneration. Toxicology and Applied Pharmacology. 2015;**282**:68-76. DOI: 10.016/j.taap.20141.007

[81] Basile JR, Binmadi NO, Zhou H, Yang Y, Paoli A, Proia P. Supraphysiological doses of performance enhancing anabolic-androgenic steroids exert direct toxic effects on neuron-like cells. Frontiers in Cellular Neuroscience. 2013;**7**:69. DOI: 10.3389/fncel.2013.00069

[82] Gervais FG, Xu D, Robertson GS, Vaillancourt JP, Zhu Y, Huang JQ, LeBlanc A, Smith D, Rigby M, Shearman MS, Clarke EE. Involvement of caspases in proteolytic cleavage of Alzheimer's amyloid-β precursor protein and amyloidogenic Aβ peptide formation. Cell. 1999;**97**:395-406. DOI: 10.1016/S0092-8674(00)80748-5

[83] Mattson MP, Chan SL. Calcium orchestrates apoptosis. Nature Cell Biology. 2003;**5**:1041-1043. DOI: 10.1038/ncb1203-1041

[84] Herceg Z, Wang ZQ. Functions of poly(ADP-ribose) polymerase (PARP) in DNA repair, genomic integrity and cell death. Mutation Research. 2001;**477**:97-110. DOI: 10.1016/S0027-5107(01)00111-7

[85] Cunningham RL, Giuffrida A, Roberts JL. Androgens induce dopaminergic neurotoxicity via caspase-3-dependent activation of protein kinase Cδ. Endocrinology. 2009;**150**:5539-5548. DOI: 10.1210/en.2009-0640

[86] Bowles DK, Maddali KK, Dhulipala VC, Korzick DH. PKCdelta mediates anti-proliferative, pro-apoptic effects of testosterone on coronary smooth muscle. American Journal of Physiology Cell Physiology. 2007;**293**:C805-C813. DOI: 10.1152/ajpcell.00127.2007

[87] Kaul S, Kanthasamy A, Kitazawa M, Anantharam V, Kanthasamy AG. Caspase-3 dependent proteolytic activation of protein kinase Cδ mediates and regulates 1-methyl-4-phenylpyridinium (MPP+)-induced apoptotic cell death in dopaminergic cells: Relevance to oxidative stress in dopaminergic degeneration. European Journal of Neuroscience. 2003;**18**:1387-1401. DOI: 10.1046/j.1460-9568.2003.02864.x

[88] Anantharam V, Kitazawa M, Wagner J, Kaul S, Kanthasamy AG. Caspase-3-dependent proteolytic cleavage of protein kinase Cδ is essential for oxidative stress-mediated dopaminergic cell death after exposure to methylcyclopentadienyl manganese tricarbonyl. Journal of Neuroscience. 2002;**22**:1738-1751

[89] Conboy L, Foley AG, O'Boyle NM, Lawlor M, Gallagher HC, Murphy KJ, Regan CM. Curcumin-induced degradation of PKCδ is associated with enhanced dentate NCAM PSA expression and spatial learning in adult and aged Wistar rats. Biochemical Pharmacology. 2009;**77**:1254-1265. DOI: 10.1016/j.bcp.2008.12.011

[90] Fortunato RS, Ferreira AC, Hecht F, Dupuy C, Carvalho DP. Sexual dimorphism and thyroid dysfunction: A matter of oxidative stress? Journal of Endocrinology. 2014;**221**:R31-R40. DOI: 10.1530/JOE-13-0588

[91] Carvalho DP, Dupuy C. Role of the NADPH oxidases DUOX and NOX4 in thyroid oxidative stress. European Thyroid Journal. 2013;**2**:160-167. DOI: 10.1159/000354745

[92] Charvin D, Vanhoutte P, Pagès C, Borrelli E, Borelli E, Caboche J. Unraveling a role for dopamine in Huntington's disease: The dual role of reactive oxygen species and D2 receptor stimulation. Proceedings of National Academy of Sciences USA. 2005;**102**:12218-12223. DOI: 10.1073/pnas.0502698102

[93] Floyd RA. Neuroinflammatory processes are important in neurodegenerative diseases: An hypothesis to explain the increased formation of reactive oxygen and nitrogen species as major factors involved in neurodegenerative disease development. Free Radical Biology and Medicine. 1999;**26**:13461355. DOI: 10.1016/S0891-5849(98)00293-7

[94] Frankenfeld SP, Oliveira LP, Ortenzi VH, Rego-Monteiro IC, Chaves EA, Ferreira AC, Leitão AC, Carvalho DP, Fortunato RS. The anabolic androgenic steroid nandrolone decanoate disrupts redox homeostasis in liver, heart and kidney of male Wistar rats. PLoS One. 2014;**9**:e102699. DOI: 10.1371/journal.pone.0102699

[95] Holmes S, Abbassi B, Su C, Singh M, Cunningham RL. Oxidative stress defines the neuroprotective or neurotoxic properties of androgens in immortalized female rat dopaminergic neuronal cells. Endocrinology. 2013;**154**:4281-4292. DOI: 10.1210/en.2013-1242

[96] De Barros S, Dehez S, Arnaud E, Barreau C, Cazavet A, Perez G, Galinier A, Casteilla L, Planat-Bénard V. Aging-related decrease of human ASC angiogenic potential is reversed by hypoxia preconditioning through ROS production. Molecular Therapy. 2013;**21**:399-408. DOI: 10.1038/mt.2012.213

[97] Sedlic F, Sepac A, Pravdic D, Camara AK, Bienengraeber M, Brzezinska AK, Wakatsuki T, Bosnjak ZJ. Mitochondrial depolarization underlies delay in permeability transition by preconditioning with isoflurane: Roles of ROS and Ca^{2+}. American Journal of Physiology Cell Physiology. 2010;**299**:C506-C515. DOI: 10.1152/ajpcell.00006.2010

[98] Prentice H, Modi JP, Wu JY. Mechanisms of neuronal protection against excitotoxicity, endoplasmic reticulum stress, and mitochondrial dysfunction in stroke and neurodegenerative diseases. Oxidative Medicine and Cellular Longevity. 2015;**2015**:964518. DOI: 10.1155/2015/964518

[99] Orlando R, Caruso A, Molinaro G, Motolese M, Matrisciano F, Togna G, Melchiorri D, Nicoletti F, Bruno V. Nanomolar concentrations of anabolic-androgenic steroids amplify excitotoxic neuronal death in mixed mouse cortical cultures. Brain Research. 2007;**1165**:21-29. DOI: 10.1016/j.brainres.2007.06.047

[100] Rossbach ULW, Steensland P, Nyberg F, Le Grevès P. Nandrolone-induced hippocampal phosphorylation of NMDA receptor subunits and ERKs. Biochemical Biophysics Research Communication. 2007;**357**:1028-1033. DOI: 10.1016/j.bbrc.2007.04.037

[101] Carrillo M, Ricci LA, Melloni RH. Developmental and withdrawal effects of adolescent AAS exposure on the glutamatergic system in hamsters. Behavioral Neuroscience. 2011;**125**:452-464. DOI: 10.1037/a0023475

[102] Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. Science. 2002;**297**:353356. DOI: 10.1126/science.1072994

[103] Vetrivel KS, Thinakaran G. Membrane rafts in Alzheimer's disease beta-amyloid production. Biochimica et Biophysica Acta. 2010;**1801**:860-867. DOI: 10.1016/j.bbalip.2010.03.007

[104] Lewczuk P, Kamrowski-Kruck H, Peters O, Heuser I, Jessen F, Popp J, et al. Soluble amyloid precursor proteins in the cerebrospinal fluid as novel potential biomarkers of Alzheimer's disease: A multicenter study. Molecular Psychiatry. 2010;**15**:138-145. DOI: 10.1038/mp.2008.84

[105] Jin M, Shepardson N, Yang T, Chen G, Walsh D, Selkoe DJ. Soluble amyloid beta-protein dimers isolated from Alzheimer cortex directly induce Tau hyperphosphorylation and neuritic degeneration. Proceedings of National Academy of Sciences United States of America. 2011;**108**:5819-5824. DOI: 10.1073/pnas.1017033.108

[106] Danysz W, Parsons CG. Alzheimer's disease, β-amyloid, glutamate, NMDA receptors and memantine - Searching for the connections. British Journal of Pharmacology. 2012;**167**:324-352. DOI: 10.1111/j.1476-5381.2012.02057.x

[107] Caraci F, Pistarà V, Corsaro A, Tomasello F, Giuffrida ML, Sortino MA, Nicoletti F, Copani A. Neurotoxic properties of the anabolic androgenic steroids nandrolone and methandrostenolone in primary neuronal cultures. Journal of Neuroscience Research. 2011;**89**:592-600. DOI: 10.1002/jnr.22578

[108] Moffat SD, Zonderman AB, Metter EJ, Kawas C, Blackman MR, Harman SM, Resnick SM. Free testosterone and risk for Alzheimer disease in older men. Neurology. 2004;**62**:188-193. DOI: 10.1212/WNL.62.2.188

[109] Cherrier MM, Matsumoto AM, Amory JK, Asthana S, Bremner W, Peskind ER, Raskind MA, Craft S. Testosterone improves spatial memory in men with Alzheimer disease and mild cognitive impairment. Neurology. 2005;**64**:2063-2068. DOI: 10.1212/01.WNL.0000165995.98986.F1

[110] Tan RS, Pu SJ. A pilot study on the effects of testosterone in hypogonadal aging male patients with Alzheimer's disease. Aging Male. 2003;**6**:13-17. DOI: 10.1080/tam.6.1.13.17

[111] Hildebrandt T, Langenbucher JW, Flores A, Harty S, Berlin H. The influence of age of onset and acute anabolic steroid exposure on cognitive performance, impulsivity, and aggression in men. Psychology of Addictive Behaviors. 2014;**28**:1096-1104. DOI: 10.1037/a0036482

[112] Kanayama G, Kean J, Hudson JI, Pope HG. Cognitive deficits in long-term anabolic-androgenic steroid users. Drug and Alcohol Dependence. 2013;**130**: 208-214. Available from: http://www.sciencedirect.com/science/article/pii/S0376871612004498

[113] Rivas-Arancibia S, Vazquez-Pereyra F. Hormonal modulation of extinction responses induced by sexual steroid hormones in rats. Life Sciences. 1994;**54**:PL363-PL367. Available from: http://linkinghub.elsevier.com/retrieve/pii/0024320594900361

[114] Vázquez-Pereyra F, Rivas-Arancibia S, Castillo AL-D, Schneider-Rivas S. Modulation of short term and long term memory by steroid sexual hormones. Life Sciences.

1995;**56**:PL255-PL260. Available from: http://linkinghub.elsevier.com/retrieve/pii/002432059500067G

[115] Magnusson K, Hånell A, Bazov I, Clausen F, Zhou Q, Nyberg F. Nandrolone decanoate administration elevates hippocampal prodynorphin mRNA expression and impairs Morris water maze performance in male rats. Neuroscience Letters. 2009;**467**:189-193

[116] Tanehkar F, Rashidy-Pour A, Vafaei AA, Sameni HR, Haghighi S, Miladi-Gorji H, et al. Voluntary exercise does not ameliorate spatial learning and memory deficits induced by chronic administration of nandrolone decanoate in rats. Hormone and Behavior. 2013;**63**:158-165. DOI: 10.1016/j.yhbeh.2012.10.003

[117] Kouvelas D, Pourzitaki C, Papazisis G, Dagklis T, Dimou K, Kraus MM. Nandrolone abuse decreases anxiety and impairs memory in rats via central androgenic receptors. International Journal of Neuropsychopharmacology. 2008;**11**:925-934. DOI: 10.1017/S1461145708008 754

[118] Scoville WB, Milner B. Loss of recent memory after bilateral hippocampal lesions. Journal of Neurology, Neurosurgery Psychiatry. 1957;**20**:11-21. Available from: http://dx.doi.org/10.1136/jnnp.20. 1.11

[119] Tulving E, Markowitsch HJ. Episodic and declarative memory: Role of the hippocampus, Hippocampus. 1998;**8**:198-204. DOI:10.1002/(SICI)1098-1063(1998)8:3<198::AID-HIPO2>3.0.CO;2-G

[120] Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj EK, Jones PK, Ghanbari H, Wataya T, Shimohama S, Chiba S, Atwood CS, Petersen RB, Smith MA. Oxidative damage is the earliest event in Alzheimer disease. Journal of Neuropathology and Experimental Neurology. 2001;**60**:759-767. DOI: 10.1093/jnen/60.8.759

[121] Simerly RB, Swanson LW, Chang C, Muramatsu M. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: An in situ hybridization study. Journal of Comparative Neurology. 1990;**294**:76-95. DOI: 10.1002/cne.902940107

[122] Beyenburg S, Watzka M, Clusmann H, Blümcke I, Bidlingmaier F, Elger CE, Stoffel-Wagner B. Androgen receptor mRNA expression in the human hippocampus. Neuroscience Letters. 2000;**294**:25-28. DOI: 10.1016/S0304-3940(00)01542-1

[123] Brännvall K, Bogdanovic N, Korhonen L, Lindholm D. 19-Nortestosterone influences neural stem cell proliferation and neurogenesis in the rat brain. European Journal of Neuroscience 2005;**21**:871-878. DOI: 10.1111/j.1460-9568.2005.03942.x

[124] Witter MP, Amaral DG. Entorhinal cortex of the monkey: V. Projections to the dentate gyrus, hippocampus, and subicular complex. Journal of Comparative Neurology. 1991;**307**:437-459. DOI: 10.1002/cne.903070308

[125] Kee N, Teixeira CM, Wang AH, Frankland PW. Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus. Nature Neuroscience. 2007;**10**:355-362. DOI: 10.1038/nn1847

[126] Xavier GF, Oliveira-Filho FJB, Santos AMG. Dentate gyrus-selective colchicine lesion and disruption of performance in spatial tasks: Difficulties in "place strategy" because of a lack of flexibility in the use of environmental cues? Hippocampus. 1999;**9**:668-681. DOI: 10.1002/(SICI)1098-1063(1999)9:6<668::AID-HIPO8>3.0.CO;2-9

[127] Rodríguez JJ, Jones VC, Tabuchi M, Allan SM, Knight EM, LaFerla FM, Oddo S, Verkhratsky A. Impaired adult neurogenesis in the dentate gyrus of a triple transgenic mouse model of Alzheimer's disease. PLoS One. 2008;**3**:e2935. DOI: 10.1371/journal.pone.0002935

[128] Chun XL, Jiang J, Qi GZ, Xin JZ, Wang W, Zhi JZ, Han X, Dong YZ. Voluntary exercise-induced neurogenesis in the postischemic dentate gyrus is associated with spatial memory recovery from stroke. Journal of Neuroscience Research. 2007;**85**:1637-1646. DOI: 10.1002/jnr.21317

[129] Pereira AC, Huddleston DE, Brickman AM, Sosunov AA, Hen R, McKhann GM, Sloan R, Gage FH, Brown TR, Small AS. An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. Proceedings of National Academy of Sciences USA. 2007;**104**:5638-5643. DOI: 10.1073/pnas.0611721104

[130] Novaes Gomes FG, Fernandes J, Vannucci Campos D, Cassilhas RC, Viana GM, D'Almeida V, de Moraes Rêgo MK, Buainain PI, Cavalheiro EA, Arida RM, Gomes F, Fernandes J, Campos D. The beneficial effects of strength exercise on hippocampal cell proliferation and apoptotic signaling is impaired by anabolic androgenic steroids, Psychoneuroendocrinology. 2014;**50**:106-117. DOI: 10.1016/j.psyneuen.2014.08.009