Neurotoxicity by Synthetic Androgen Steroids: Oxidative Stress, Apoptosis, and Neuropathology: A Review

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Abstract: Anabolic-androgenic steroids (AAS) are synthetic substances derived from testosterone that are largely employed due to their trophic effect on muscle tissue of athletes at all levels. Since a great number of organs and systems are a target of AAS, their adverse effects are primarily on the following systems: reproductive, hepatic, musculoskeletal, endocrine, renal, immunological, infectious, cardiovascular, cerebrovascular, and hematological. Neuropsychiatric and behavioral effects as a result of AAS abuse are well known and described in the literature. Mounting evidence exists suggesting that in addition to psychiatric and behavioral effects, non-medical use of AAS carries neurodegenerative potential. Although, the nature of this association remains largely unexplored, recent animal studies have shown the recurrence of this AAS effect, ranging from neurotrophin unbalance to increased neuronal susceptibility to apoptotic stimuli.

Experimental and animal studies strongly suggest that apoptotic mechanisms are at least in part involved in AAS-induced neurotoxicity. Furthermore, a great body of evidence is emerging suggesting that increased susceptibility to cellular oxidative stress could play a pivotal role in the pathogenesis of many neurodegenerative disorders and cognitive impairment. As in other drug-evoked encephalopathies, the key mechanisms involved in AAS – induced neuropathology could represent a target for future neuroprotective strategies. Progress in the understanding of these mechanisms will provide important insights into the complex pathophysiology of AAS-induced neurodegeneration, and will pave the way for forthcoming studies. Supplementary to abandoning the drug abuse that represents the first step in reducing the possibility of irreversible brain damage in AAS abusers, neuroprotective strategies have to be developed and implemented in future.

Keywords: Androgen-anabolic steroids, apoptosis, biochemical mechanisms, excitotoxic neuronal death, neurotrophin unbalance, neuroprotective strategies, neurotoxicity, oxidative-stress.

INTRODUCTION

Anabolic-androgenic steroids (AAS) are synthetic substances derived from testosterone (Fig. **1**) that are employed for their trophic effect on muscle tissue, with a net result in increased muscle mass and strength. These effects, in conjunction with the neurostimulatory ones, may explain the large prevalence of AAS among athletes at all levels [1-7]. Athletes, and namely bodybuilders and weightlifters, are the main users of these substances [1, 6]. Individuals who desire a lean appearance and muscular appearance are also implicated in this [2, 3, 7, 8-18].
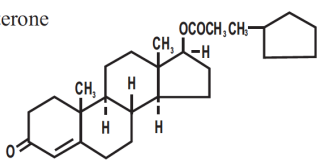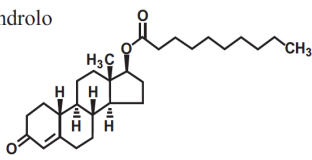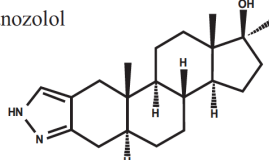
Although the use of anabolic steroids for cosmetic purposes is incorrectly thought to be relatively harmless, contrarily, anabolic steroids are harmful to health [4, 19-21]. In fact, their consumption can trigger a series of adverse side effects on the body. It is generally believed that side effects of AAS could develop only as a result of long-term use [22]. However, acute adverse effects have also been described, primarily consisting of headaches, fluid retention, gastrointestinal irritation, diarrhoea, abdominal pain, jaundice, menstrual abnormalities, and hypertension. The chronic effects of AAS abuse aside from the neuro-psychiatric and behavioral effects include a wide range of somatic consequences. Many organs and systems are targets of AAS action; consequently, AAS may exert negative effects on reproductive, hepatic, musculoskeletal, endocrine, renal, immunologic, cardiovascular, cerebrovascular, and hematological systems [5, 23-27].

Neuropsychiatric and behavioral effects of AAS abuse are well known and described in the literature [9, 12, 28-52]. In rodents, long-term administration of certain AAS induces behavioral and neurochemical changes [44, 53-58], which may resemble similar behavioral modifications observed in AAS abusers.

However, the neurodegenerative effects of long term AAS abuse are part of not yet evident phenomenon in which the negative effects of these drugs remain clinically silent during the young age. Most of the world's illicit AAS users

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AAS Class	I	II	III
	C-17 Esterification	**19-Nortestosterone**	**C-17 Alkylation**
Substitution	Long-chain ester	No methyl group at C-19; may also have C-17 esters	Alkyl moiety at C-17
Examples	• Testosterone cypionate • Testosterone propionate • Methenolone	• Nandrolone decanoate • Trenbolone	• Stanozolol • 17α-Methyltestosterone • Methandrostenolone • Oxymetholone
Structure	Testosterone	Nandrolo	Stanozolol

Fig. (1). The three major classes of anabolic androgen steroids (modified from Oberlander, J.G.; Henderson, L.P, 2012, cited sub 51).

are still under the age of 50, too early for clear symptomatic manifestations (cognitive or motor deficits) of possible neurotoxic effects. [59]. Recently, Kanayama *et al*. [59] reported that visio-spatial memory of long-term AAS users was significantly worse than in AAS non-users. Moreover the same Authors reported that visio-spatial performance showed a significant negative correlation with total lifetime AAS dosing [59]. These observations are in line with the experimental data reported by Pierretti *et al*. [60] who demonstrated that rats administered with supraphysiologic AAS doses showed spatial memory deficits.

There is growing evidence that non-medical use of AAS has a neurodegenerative potential. Although the nature of this effect is still largely not clarified, recent animal studies have shown the recurrence of neurotoxic effects of AAS, ranging from neurotrophin unbalance to increased neuronal susceptibility to apoptotic stimuli [61].

The current paper aims to investigate the neurotoxicity related to AAS abuse and the underlying hypothesized mechanisms.

AAS NEUROACTIVITY

For a long time, steroid hormones have been demonstrated to control sexual differentiation of the brain, reproduction, behavior, memory, *etc*. [62-64].

A mass of studies demonstrated that nervous system is a target for two different pools of steroids: steroid hormones produced in the peripheral glands, and neurosteroids originating directly from the nervous system [65].

Steroids are biologically active at classical androgen receptors (ARs), albeit with significantly different activities [66-69].

Once bound by their ligands, ARs act as nuclear transcription factors eliciting the expression of genes under control of steroid-response elements (SRE), within a time course of hours following AAS binding to ARs. This pathway is supposed to be at the basis of medium- and long-term effects of steroid hormones (such as the regulation of hypophyseal hormones secretion, or sexual differentiation of brain circuits). However, the well known short-term effects of steroids led to hypothesize the existence of other receptors (the so-called non-classical steroid receptors) located within the membrane. The following receptors also act in the same manner: γ-Aminobutyric acid (GABA) type A and B (GABA-A receptor, GABA-B receptor), serotonin type 3 (5-HT3), *N*-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate receptor, and an atypical intracellular receptor like the sigma 1 [70-73].

As widely demonstrated in the literature, aggression, anxiety, and reproductive behaviors involving GABAergic transmission are mainly affected by AAS use in both human abusers and animal models [44, 74].

The central region of the medial preoptic area (mPOA), the medial preoptic nucleus (MPN), is characterized by a dense presence of GABAergic neurons [75, 76] and GABA A receptors [56, 77], as well as high levels of ARs, estrogen receptors (ERs), and aromatase, consistent with the high steroid-affinity of this region [56].

In an experimental animal model, it was demonstrated that remarkable modifications in the levels of selective GABA A receptor subunit mRNAs, depending upon the dose of AAS, and on the age and the sex of the animals [53] were induced by chronic administration of AAS.

AAS can also be aromatized to estrogens and interface with both estrogen receptor alpha and beta (ERα, ERβ) [68, 78-84]. Moreover, AAS can also act indirectly altering signaling *via* ER by their ability to allosterically inhibit aromatase [78, 85], through interactions with a non-AR/ER microsomal binding site [86] as well as allosteric modulation of ion channels [87-89]. Furthermore, the AAS induced decrease of endogenous neurosteroids biosynthesis, may

explain the non-receptor-mediated effects on both aggression and anxiety [78, 85, 90-91].

Conclusively, the classical AAS mechanisms of action imply steroid binding to the ARs. However, the kaleidoscopic mechanisms of AAS activity are further complicated by the fact that some steroids involve non-classical and non-genomic mechanism responses [92-94] (Fig. **2**).

There is a growing body of evidence suggesting that non-genomic effects of AAS act in concert with genomic effects. Rapid, non - genomic effects of AAS differ from genomic ones in: 1) faster onset (seconds to minutes), 2) insensitivity to inhibition of RNA and protein synthesis, 3) effects
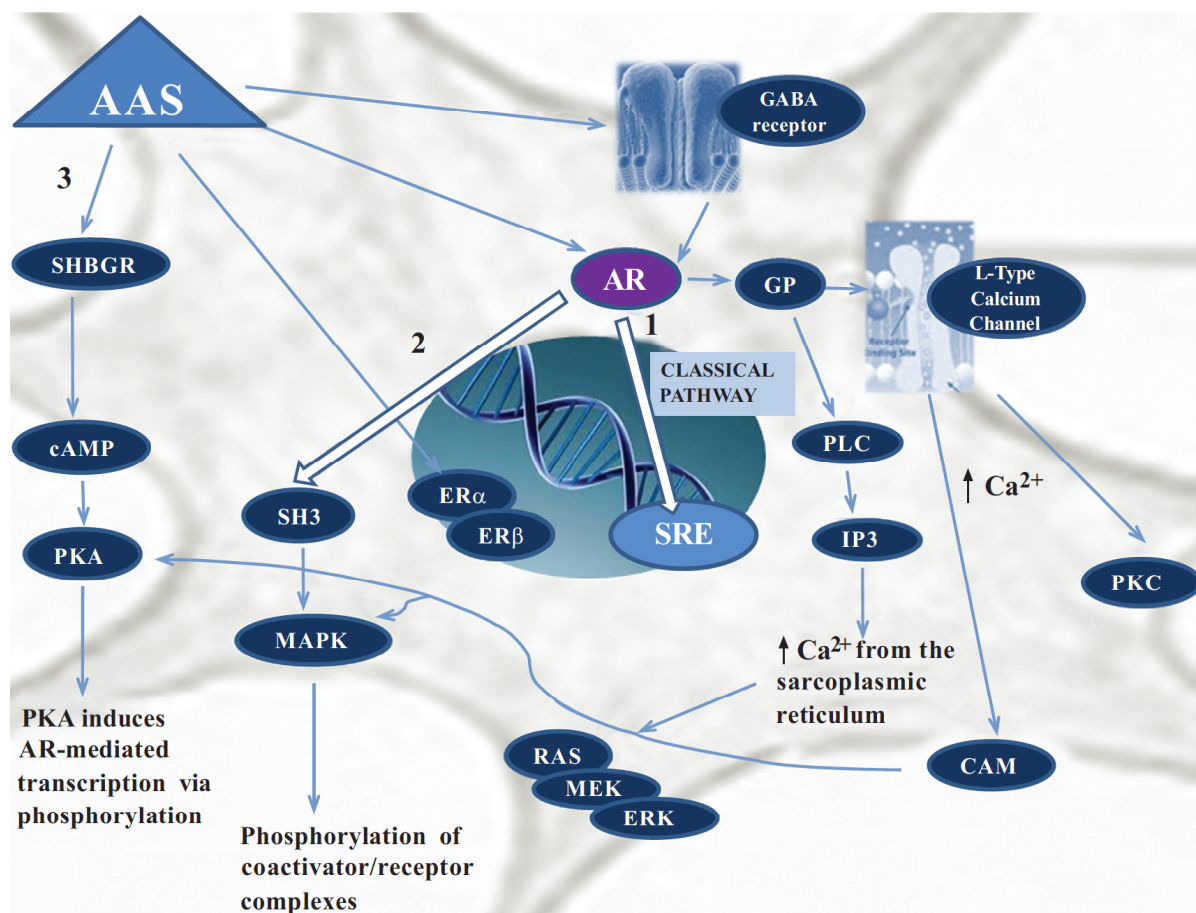
Fig. (2). The different mechanisms of AAS neurotoxicity. In the classical pathway (1), the androgen freely passes through the membrane bi-layer and binds cytoplasmic Androgen Receptor (AR); after translocation to the nucleus bound AR binds to Steroid-Response Elements (SRE), stimulating gene transcription. Bound AR also interacts with Src Homology Domain 3 (SH3) of the tyrosine kinase c-Src to activate the Mitogen-Activated Protein Kinase (MAPK) pathway and induces gene transcription *via* phosphorylation of coactivator/receptor complexes (2). The androgen binds to Steroid Hormone Binding Globulin (SHBG) activating the SHBG Receptor (SHBGR) and leading to an increase in Protein Kinase A (PKA) activity. PKA may influence AR-mediated transcription *via* alteration of phosphorylation status of AR and AR coregulators (3). AAS can be also metabolized to estrogens, interacting not only with AR but also with Estrogen Receptors (ERα and ERβ) to regulate gene transcription. These interaction can result in change in GABAA receptor subunit gene expression. AAS also induce directly changes in GABAergic signaling altering gene expression. On the right the non-genomic androgen action *via* changes in intracellular ion concentrations and membrane fluidity is represented. Androgen interacts with a membrane associated AR leading to the activation of L-type calcium channels through some type of inhibitory g-protein (GP). This increase in intracellular calcium activates Protein Kinase C (PKC), and activates *via* calmodulin (CAM) PKA and MAPK pathway. The modulation of GP may also activate Phospholipase C (PLC) resulting in increases in inositol 1,4,5-thriphosphate (IP3) and consequently in release of intracellular calcium stores from the sarcoplasmic reticulum and in activation of the RAS/MEK/ERK pathway (MEK=MAPK/ERK kinase, ERK=extracellular-signal regulated kinase).

produced by steroids unable to access the nucleus (either covalently linked to membrane impermeable macro-molecules or in cells lacking a nucleus), and 4) not usually blocked by classical antagonists due to different steroidal specificity from classical cognate nuclear receptors [95]. As for other steroids, non-genomic AAS effects include a broad spectrum of intracellular pathways such as the activation of membrane bound receptors, triggering of downstream pathways involving protein kinases and phosphatases, mobilization of intracellular Ca^{2+}, and SRE-independent changes in transcription [95]. Androgens can bind to receptors in or around the plasma membrane, activate cell-signaling pathways, and regulate responses on a time scale of seconds or minutes [93]. This effect has been demonstrated in several kinds of cells, including osteoblasts, platelets, skeletal muscle cells, cardiac myocytes and neurons.

A rapid (seconds to minutes) change in $[Ca^{2+}]i$ is the main non-genomic effect of androgen exposure [96-103].

The versatility of Ca^{2+} as a second messenger is implicated in a variety of pathophysiological processes, such as cell proliferation, apoptosis, necrosis, motility, and gene expression [93, 103, 104]. Neurons seem to be particularly sensitive to Ca^{2+} oscillations [102] because Ca^{2+} controls pivotal neural processes including synaptic plasticity [105], exocytosis [106], gene expression [107, 108], bioenergetics [109], autophagy [110], and apoptosis [111].

Moreover, androgen induced $[Ca^{2+}]i$ rise may regulate ARs activation since increased $[Ca^{2+}]i$ levels stimulate the binding of androgens to ARs [112]. In addition, androgens can activate calcium dependent kinase pathways, such as extracellular signal-regulated kinase (ERK) or Src, which could phosphorylate the ARs and enhance its activity [113, 114]. The increase in $[Ca^{2+}]i$ following treatment with Ca^{2+} ionophores or inhibitors of Ca^{2+}-ATPase have also been found to reduce ARs expression [115]. Conclusively, Ca^{2+} could act as a pivotal link between non-genomic and genomic AAS signaling [103].

AAS NEUROTOXICITY

At physiological levels, androgens influence neuronal differentiation, neuroprotection, neuronal survival, and development [116-118], likely through the classic, slow AR pathway.

Estrada *et al.* demonstrated that following the administration of physiological doses of testosterone, rapid intracellular Ca^{2+} increases in neuroblastoma cells are evoked, leading to neurite outgrowth [119], which is essential in neuronal differentiation [120].

Neurobehavioral changes like hyperexcitability and supra-aggressive nature observed following the administration of large doses of androgen could represent the clinical expression of neuronal damage resulting from exposure to high concentrations of AAS [9, 12, 28-52].

The mechanisms of these deleterious neuropathological effects of AAS have not yet been completely elucidated, and are still largely unexplored; however evidence has shown the recurrence of neurodegenerative and neurotoxic potential of these compounds, ranging from neurotrophin unbalance to increased neuronal susceptibility to apoptotic stimuli [61].

The expression 'apoptosis' was coined by Kerr *et al.* [121] to describe peculiar morphological aspects of cell death. In apoptosis, cells appear rounded – up, pseudopods are retracted, cellular volume is reduced (pyknosis), chromatin is condensed, and nuclei are fragmented (karyorrhexis). Other typical findings of apoptosis are little or no ultrastructural modifications of cytoplasmic organelles, plasma membrane blebbing, and engulfment by resident phagocytes (*in vivo*) [122] (Fig. **3**).

The mechanisms leading to apoptotic death are very complex. Extrinsic apoptosis is a caspase – dependent cell death subroutine elicited by extracellular stress signals that are sensed and propagated by specific transmembrane receptors. It can be initiated by the binding of lethal ligands, such as FAS/CD95 ligand (FASL/CD95L), tumor necrosis factor α (TNFα) and TNF superfamily member 10 (TNFSF10, also known as TNF-related apoptosis inducing ligand, TRAIL), to various death receptors (i.e., FAS/CD95, TNFα receptor 1 (TNFR1), and TRAIL receptor (TRAILR) 1–2, respectively [123].

The apoptotic death can be triggered by many different intracellular stress conditions, including DNA damage, oxidative stress, cytosolic Ca2þ overload, mild excitotoxicity (related to glutamate receptor overstimulation in the nervous system), accumulation of unfolded proteins in the endoplasmic reticulum (ER), and many others (the so-called "intrinsic apoptosis") [123]. The initiating stimuli and the signaling cascade involved in intrinsic apoptosis are strictly linked to a mitochondrion-centered control mechanism.

It is well known that AAS can exert apoptotic stimuli in different tissue and organs, therefore representing a pivotal mechanism in AAS – induced toxicity [124-135]. Growing evidence is emerging that apoptotic mechanisms are at least in part, also involved in AAS induced neurotoxicity.

Estrada *et al.* [136] firstly showed that the treatment of neuroblastoma cells with elevated concentrations of testosterone induces a decrease in cell viability by activation of an apoptotic cell death program, as demonstrated by increased numbers of annexin V-positive cells, DNA fragmentation, and caspase activation. The hypothesis put forward was that elevated testosterone alters InsP3R (Inositol trisphosphate receptor) type 1-mediated intracellular Ca^{2+} signaling, and that the prolonged Ca^{2+} signals lead to apoptosis [136].

Orlando *et al.* [137] studied the effect of some AAS (testosterone, nandrolone, stanozolol, and gestrinone) on excitotoxic neuronal death induced by N-methyl-d-aspartate (NMDA) in primary cultures of mouse cortical cells. The term "excitotoxicity" was coined by John Olney [138, 139] to describe a specific neuronal death pathway induced by an excessive stimulation of glutamate receptors, resulting in excessive Ca^{2+} influx through a receptor's associated ion channels [140-142]. The Authors demonstrated that only very high concentrations of testosterone were able to amplify neuronal excitotoxicity; on the contrary lower testosterone concentrations seemed to be protective. Testosterone was
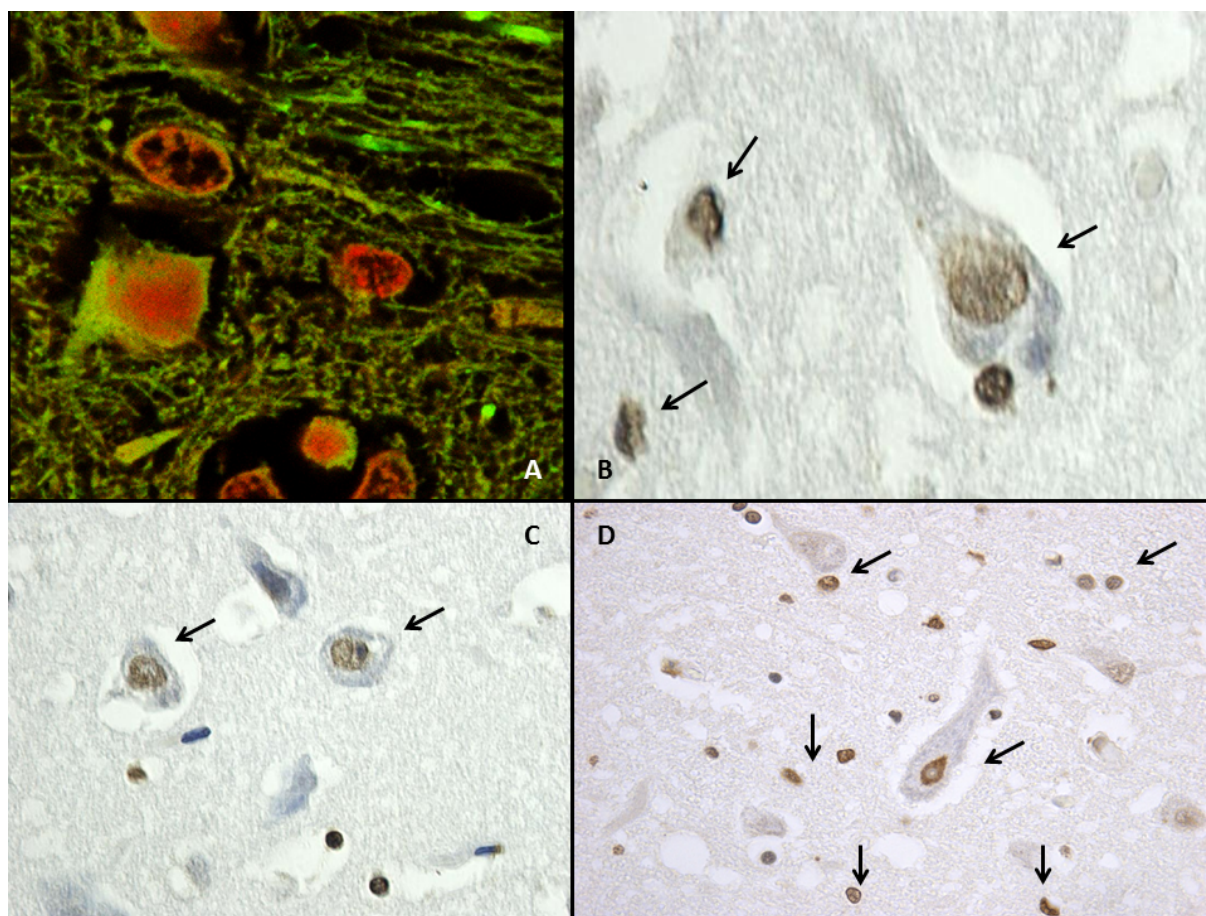
Fig. (3). Mouse brain treated with nandrolone decanoate (**A**) Confocal laser scanning microscope. Increase of apoptosis (Tunel assay) with intense positive reaction (red). (**B-C-D**) TUNEL assay revealed neuronal and glial over-expression of apoptotic nuclei (arrows) with a brownish positive reaction.

inactive at intermediate concentrations. However, the presence of aromatase inhibitors made even low concentrations of testosterone neurotoxic, therefore suggesting that aromatization of testosterone into 17β-estradiol could counter – balance its intrinsic toxicity. Contrary to testosterone, nortestosterone, stanozolol, and gestrinone-amplified NMDA toxicity at nanomolar concentrations was insensitive to aromatase inhibitors, but was abrogated by the androgen receptor antagonist, flutamide. None of the AASs was toxic in the absence of NMDA. These results led to the hypothesis that AAS increase neuronal vulnerability to excitotoxic insults and may therefore induce neuronal death observed in acute or chronic neurological diseases [137].

Other groups have experimentally demonstrated an apoptotic effect of high dosages of AAS. Cunningham *et al.* [143] showed that physiologically relevant doses of androgens induce neurotoxicity in dopaminergic neurons (N 27 cells). In this experimental model, androgens enter the cell, bind to the classical intracellular ARs, and induce oxidative stress leading to mitochondrial dysfunction. The release of cytochrome c from the mitochondria activates the apoptotic caspase cascade, promoting PKCδ (Protein kinase C-δ) activation. It has been hypothesized that androgens may induce apoptosis specifically in dopamine neurons since

testosterone-induced apoptosis was not observed in gonadotropin-releasing hormone (GnRH) neurons [143]. One group [144] demonstrated additionally that nandrolone and methandrostenolone potentiated the apoptotic trigger induced by beta-amyloid, widely involved in the pathogenesis of Alzheimer's disease. The same Authors also hypothesized that AAS abuse might support the onset and the progression of neurodegenerative diseases. Tugyan *et al.* [145], in an animal model (rats) demonstrated a net increase in apoptosis and a significant decrease in neuronal count in the parietal cortex, prefrontal cortex and hippocampal regions of the brain induced by nandrolone decanoate. In their elegant experiment on neuron – like cells (undifferentiated pheochromocytoma 12 cells), Basile *et al.* [146] demonstrated that the treatment with steroid hormones (androsterone, nandrolone, methandienone and 17-α- methyltestosterone) induced cell death through apoptotic pathways. The Authors detected the appearance of the cleaved and hence active form of caspase 3, along with the cleaved form of poly adenosine diphosphate [ADP]-ribose polymerase, (PARP), therefore suggesting that apoptosis might be a generalized response to high concentrations of steroids. The obtained results demonstrated that AAS-induced apoptosis in neuron-like differentiated pheocromocytoma cell line PC12 [146]. Finally, as it was noted that the effects on cells following AAS treatment were

delayed, the Authors speculated that these hormones might exert their effects by acting on AR-mediated genomic pathway [146] and might therefore alter gene transcription [147].

The complexity of the mechanisms of AAS induced neurotoxicity implicates oxidative stress since apoptosis itself can be induced by oxidative stress [148] (Fig. **4**).

A redox system imbalance with an excess of reactive oxygen species (ROS) and reactive nitrogen species (RNS) can contribute to neuronal cell injury and death [149-153] and has been associated with apoptosis [123, 154-157]. The redox system may play different roles in apoptosis. Protein oxidation may essentially influence the gene expression

necessary for the signals leading to apoptosis. Caspase activation, DNA binding of several transcription factors, and cytoskeletal alterations in cells undergoing apoptosis may directly or indirectly be affected by oxidative events [148].

In experimental models of neurodegenerative diseases, excessive generation of ROS such as superoxide anion (0_2^-), and RNS such as nitric oxide (NO) can contribute to neuronal cell injury and death [141, 153]. NO derived from iNOS also play a significant role in the pathogenesis of several neurodegenerative diseases such as Alzehimer's Disease (AD), Parkinson's Disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and HIV-associated neurocognitive disorder, through the activation of
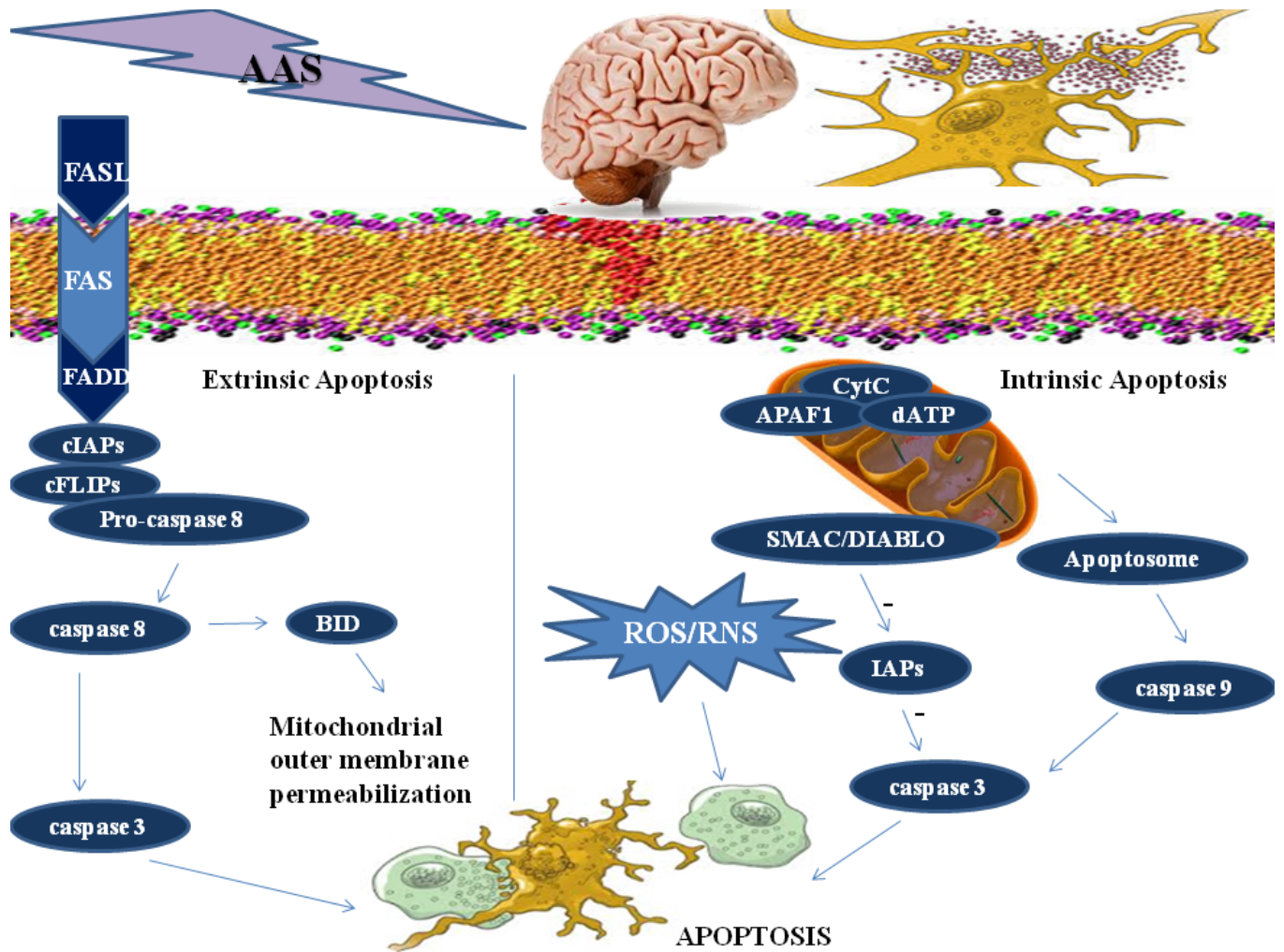
Fig. (4). A schematic illustration of the complex mechanisms leading to neuronal death. Extrinsic apoptosis is a caspase – dependent cell death subroutine that is initiated by the binding of lethal ligands, such as FAS/CD95 ligand (FASL/CD95L) to death receptor FAS/CD95; the complex recruit FAS-associated protein with a death domain (FADD), cellular inhibitor of apoptosis proteins (cIAPs), c-FLIPs and pro-caspase 8. This supramolecular platform controls the activation of caspase-8, that can directly trigger the caspase cascade by mediating the proteolytic maturation of caspase-3, or stimulate mitochondrial outer membrane permeabilization by cleaving the BH3 interacting-domain (BID). In the intrinsic apoptosis the multiple intracellular stress conditions lead to a mitochondrion-centered control mechanisms. When lethal signals prevail, mitochondrial outer membrane permeabilization occurs and leads to mitochondrial transmembrane potential dissipation and arrest of mitochondrial ATP synthesis. The respiratory chain gets uncoupled, leading to reactive oxygen species (ROS) and reactive nitrogen species (RNS) production. Cytochrome C (CytC), together with the cytoplasmic adaptor protein APAF1 and dATP, create the apoptosome, that triggers the caspase 9-caspase 3 proteolytic cascade. Direct IAP-binding protein with low pl (DIABLO, also known as second mitochondria-derived activator of caspases, SMAC) induces caspase activation.

glial cells (astrocytes and microglia) induced by a plethora of neuroinflammatory and neurodegenerative stimuli [158, 159]. Activated glial cells result in expression of iNOS and production of high levels of NO [158, 159]. Oxidative stress has been shown to initiate the apoptotic pathway also in animal models of PD [160-163].

A great body of evidence is emerging that increased susceptibility to cellular oxidative stress could play a pivotal role in the pathogenesis of many neurodegenerative disorders and cognitive impairment [164-169]. These findings are strongly supported by experimental data that confirm the potential neurotoxicity of oxidative stress [170-174].

Our group has pointed out the role of oxidative stress in the mechanisms of AAS-induced toxicity in various organs, such as liver, cardiovascular system and kidney [125, 175-177].

Other Authors focused on the potential dangerous link between androgens and oxidative stress in neurotoxicity. Holmes *et al.* investigated the effects of androgens under conditions of oxidative stress to determine whether androgens play a neuroprotective or neurotoxic role in dopamine neuronal function. It was discovered that androgens themselves increased mitochondrial function *via* a calcium-dependent mechanism. Androgen pre-treatment protected cells from oxidative stress-induced cell death. However, treatment with androgens after the oxidative insult increased cell death, and these effects were in part mediated by calcium influx into the mitochondria, and the negative effects of androgens were not blocked by either androgen or estrogen receptor antagonists. A membrane-associated androgen receptor was supposed to be implicated. The results of this study suggested that androgens are neuroprotective when oxidative stress levels are minimal, but when oxidative stress levels are elevated, androgens exacerbate oxidative stress damage [178]. Similar results were reported by Cunningham *et al.* [179] who demonstrated that testosterone appears to have negative consequences on brain function under conditions of elevated oxidative stress in the Caucasian race. The same group [180] demonstrated that in a preexisting oxidative stress environment, androgens can further exacerbate oxidative stress damage.

Taken together, all these studies indicate a potential role for androgens in oxidative stress – mediated neurodegeneration.

Clinical observations further support this hypothesis. Compared to women, men have a higher incidence of post ischemic stroke Parkinsonism, a neurodegenerative condition in which oxidative stress is strongly implicated in the progression of cell death [181, 182]. Moreover, at the same age, men have a greater incidence of neurodegenerative diseases such as PD than women [183-185].

However, some studies suggest a neuroprotective role for androgens [186-188]. More recently, Nguyen *et al.* hypothesized that androgen protection could be specific to apoptosis. The results of this study demonstrated that testosterone attenuated neuronal death induced by apoptotic pathways [189]. A different effect was demonstrated for the oxidative stress – induced neuronal injury: direct anti-oxidant mechanism may be not involved in neuroprotection induced by androgens since micromolar concentrations of

testosterone have no protective effects against cell death induced by the oxidative stressors [189]. However, other Authors previously reported conflicting results as they showed that in neuroblastoma cells, neuroprotective effects of testosterone could deal with the cellular antioxidant defense system [190, 191]. Other studies support a neuro-protective role for androgens, in which androgens can protect against oxidative stress damage [192, 193]. A possible mechanism for androgen-induced neuroprotection is preconditioning because androgens themselves can moderately increase oxidative stress and apoptosis [143]. These results suggest that the level of oxidative stress determines whether androgens play a positive or negative role in neuronal function [178].

The scenario of the potential neurotoxicity by AAS is further complicated by other mechanisms that have been focused by several authors.

A biochemical basis for the AAS – induced neuropsychiatric sequelae has been hypothesized from the observation that in rats administered with high doses of AAS, levels of brain-derived neurotrophic factor (BDNF) in the hippocampus (HIPP) and prefrontal cortex (PFC) are reduced by stanozolol. It also decreased the expression of low-affinity glucocorticoid receptors in the HIPP, and increased morning trough basal plasma corticosterone levels [194], and decrements of BDNF production occurring in the context of a maladaptive response to stress which may contribute to the reduced volume of the hippocampus and prefrontal cortex observed in depressive disease [195]. In rats, chronic administration of AAS induced modifications in the HPA (hypothalamic-pituitary-adrenal) axis and BDNF levels which are coherent with the current hypothesized pathophysiology of depression [194]. Tucci *et al.* [196] tested this suggestive hypothesis and examined the possible biochemical changes in different brain areas of the stanozolol-treated animals. The results reported by the authors showed that 5-HT levels decreased in all brain areas following stanozolol treatment. Moreover, the steroid differently affects dopaminergic system in the PFC and HIPP of rats whereas no significant changes were observed in the STR (striatum) or NAC (nucleus accumbens). In particular, in rats chronically administered with stanozolol reduced dopamine levels in PFC are observed, so reinforcing the view that chronic treatment with stanozolol seems to reproduce the neurochemical background of depression [196].

Finally, a neurotrophin unbalance has been proposed as an AAS neurotoxic mechanism involving the nerve growth factor (NGF), a member of the neurotrophin family that promotes the differentiation, growth, and survival of specific neuronal populations during development and in the adulthood [60, 61]. In experimental setting it was demonstrated that adult rats chronically injected with high doses of testosterone or nandrolone showed an increase in NGF levels in the hippocampus and septum [197]. In their animal model, Pieretti *et al.* found that the expression of NGF and its receptors changed with a region – specific pattern following AAS treatment. NGF levels were reduced in the basal forebrain of rats treated with nandrolone or stanozolol, suggesting that the retrograde transport of NGF from the hippocampus to the basal forebrain was impaired by AAS, This observation

DRUG-EVOKED ENCEPHALOPATHY
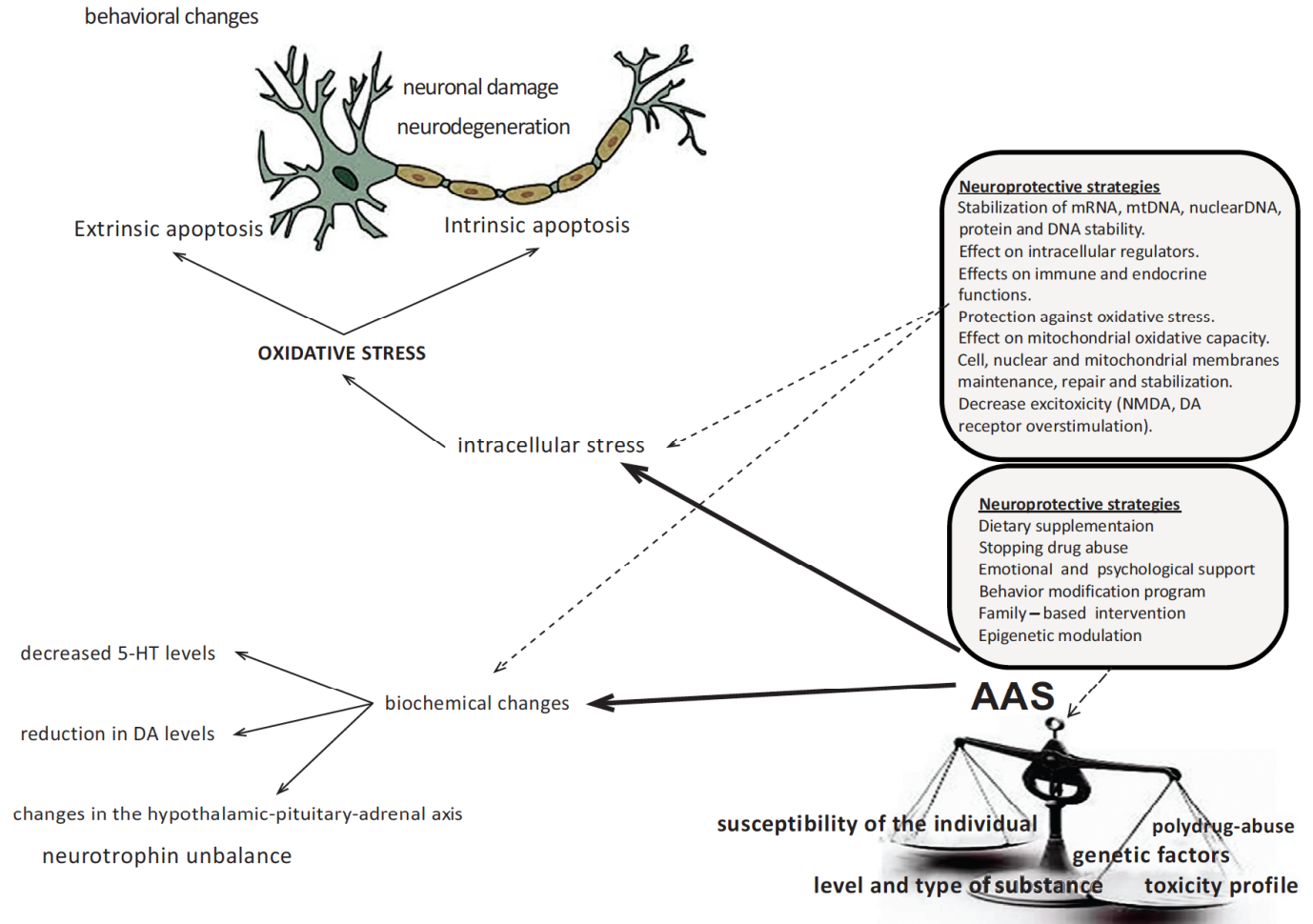
Fig. (5). The multifaceted therapeutic approach to AAS abuse evoked neuropathy focuses on "traditional" and new treatment strategies. The first strategy would be to stop the subject from the abuse. Restore normal metabolism and correct nutritional deficiencies is a further fundamental step because drug abuse may cause nutritional insufficiencies. Psychological and family – based support also play and effective role. Another potential strategy is the potential of epigenetic modulation in reducing the risk of developing the drug abuse-evoked encephalopathy. Finally, new therapeutic strategies imply targeting the genomic, metabolic, and mitochondrial dysfunction resulting in increased excitotoxicity, reduced energy production, and lowered antioxidant potential.

reinforced the hypothesis that supraphysiological doses of AAS induce neurotrophin unbalance and related behavioral disturbances [60].

Taken together, all these studies strongly support the evidence of deleterious responses of brain tissue when exposed to high doses of AAS.

Although the mechanisms of AAS effects on the brain are still unclear, some key points may be drawn.

Androgens can exert neurotoxic effects both *via* direct mechanisms including apoptosis and oxidative stress, and by intensifying neuronal excitotoxicity, a manner of neuronal death that is strongly involved in the pathophysiology of acute and chronic neurodegenerative disorders, even at low concentrations [137]. On the other hand, neuroprotective effects of androgens have been reported in the literature and seem to be related to the level of oxidative stress [178]. In

addition, the data provide evidence that suggests a different mechanism for the role of AAS in neurodegeneration and cognitive impairment related to neurotrophin unbalance and biochemical mechanisms.

CONCLUSIONS

A wide plethora of AAS side effects which mainly affect the cardiovascular system, the liver, the kidney, the musculoskeletal, and the endocrine systems have been described in the literature. In recent years, it has become more and more evident that AAS abuse may be detrimental to brain function [198].

Clinical studies demonstrated that in both adults and adolescents, AAS abuse is associated with disorders such as high level of anxiety, irritability, poor impulse control, mood fluctuation. In addition neurodegenerative potential effects of AAS abuse have been strongly suggested. However, great

difficulties exist in studying this particular kind of AAS – induced toxicity. First of all, a general limitation of human studies is due to the fact that information about the modality and doses of AAS use/abuse are often self reported and not objectively assessed [23]. Furthermore, the habit of polydrug abuse makes hardly possible to distinguish the toxic effects of AAS from those caused by other drugs [199]. Human studies mimicking the real entity of self underground administration of AAS activity are infeasible since it would be unethical to administer high doses of AAS over prolonged periods of time to assess the risks to health [19]. Finally, the susceptibility of the individuals themselves is partly dependent on genetic factors, a well known key factor in developing adverse events drug – related [200].

In this context, animal models and experimental *in vitro* studies allowing the detection of the pathological response resulting from anabolic compound administration are well consolidated approaches to detect the negative effects of AAS abuse. Assessment of how AAS exert their neurotoxic effects is a critical question that needs to be further addressed to fully understand the underlying neurobiological signaling mechanisms associated with AAS abuse, and provide new insights for therapeutic intervention. The global evidence emerging from the studies existing on the issue of AAS-induced neurodegeneration allow the affirmation that apoptosis and oxidative stress are strongly involved, and represent the major pathway for AAS neurotoxicity. It is well known that oxidative and nitrosative stresses play a key role in normal physiology and imbalances are attributed to pathology and disease. Redox signaling through oxidation and reduction reactions is a pivotal key in numerous cell signaling cascades, including those with opposing cellular consequences, proliferation, and apoptosis [201, 202]. Our current understanding of the features and the molecular targets of ROS/RNS that regulate cell-signaling pathways is limited, especially in the substance abuse field [203].

By further understanding the components of the cellular dysregulatory events induced by AAS abuse, there should be opportunities to intervene in drug – evoked pathologies, through specific therapeutic platforms. In the context of a complex and multi-faceted therapeutic approach [203, 204], the possibility of targeting redox pathways and reducing excitotoxicity and apoptosis may represent future promising therapeutic neuroprotective strategies [205, 206] (Fig. **5**).

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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