Impact of Anabolic Androgenic Steroids on Neuropeptide Systems

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Abstract: The abuse of anabolic androgenic steroids (AAS) is relatively widely spread and epidemiological studies in the western countries report a prevalence between 1-5 % among males. The impact of these steroids on the strength and muscle mass as well as many of the adverse physical effects that have been observed are well described. Several reports have also revealed severe psychological effects as results of the administration of AAS. Effects such as irritability, aggressiveness, anxiety and depression are reported to be associated with AAS abuse. The mechanistic rationales behind these effects are not well understood. Several systems are likely to be involved, including the monoamine and peptidergic systems. The aim of this review is to highlight the potential role of the neuropeptide systems in the brain with focus on how these systems are affected by repeated administration of AAS.

Keywords: Anabolic androgenic steroids, nandrolone decanoate, neuropeptide, substance P, opioids.

INTRODUCTION

Anabolic androgenic steroids (AAS) have been used by athletes for more than 50 years. However, during the 1980s, these steroids became more commonly used outside the arena of sports [1] and today, not only athletes and bodybuilders, but also adolescents and young adults not connected to sports are unfortunately often frequent steroid users. Several epidemiologic studies have been conducted in the last decade to determine the prevalence of AAS abuse among adolescents and a typical reported life-time prevalence is in the range of 1-5% among males in these studies [2-7], for a review see [8]. For example, in a study conducted in 1995, in Uppsala, Sweden, 2.7% of the male and 0.4% of the female senior high school students reported life-time use of AAS [9]. Seven years later, using an identical multiple-choice questionnaire, the reported life-time use of AAS had increased to 4.7% among the males in the first grade of senior high school [10]. In certain subpopulations, such as bodybuilders or male prisoners, the prevalence of AAS is reported to be approximately 10% or in some cases even higher [11-14].

Adolescents participating in sports still are slightly more likely to use AAS [5, 6], although other studies only observe a correlation between AAS and certain sports such as weight training [15]. Other groups characterized by low self-esteem, truancy and often bad school achievements are also more frequently using AAS [16]. Eating disorders, depressed mood and substance abuse are reported to be more frequent among AAS users [17]. Besides using anabolics in order to get larger muscles, adolescents and young males have reported using AAS in order to become brave, become intoxicated, out of curiosity or because friends do so [9, 18]. The higher prevalence of AAS use among teenagers has become a major concern. Furthermore, one should be worried about that there are studies reporting that AAS are administered in order to become more aggressive [19]. In fact, there is also a strong connection between AAS abuse and violence [20-22] and wives and girlfriends often become victims of physical abuse [23]. Criminality, such as violence and weapon offences have also been reported to associated with AAS especially when AAS is co-administered with other drugs of abuse [24].

There are numerous of different AAS on the illegal market today. Due to the limited availability of approved AAS, there also seems to be many counterfeit products on the illegal market [25]. The counterfeited products may in some cases contain other steroids and in other cases contain no drug at all [26]. In fact, 35% of the AAS analyzed in a German study did not contain the expected substances [27]. However, not knowing what is injected seems to be of little concern since AAS abusers often perceive themselves as being invulnerable. Furthermore, among adolescents who have used anabolics, 25% have shared needles [4]. Thus, AAS users might be exposed to a higher risk of attracting diseases such as human immunodeficiency virus (HIV) and hepatitis infections.

Bodybuilders and athletes usually administer the steroids in cycles 2-3 times per year, each cycle lasting 6-12 weeks. However, some steroid users also go year round in the hope for optimal results. During cycling it is often common to use 2-3 different steroids at the time, so called stacking [21, 28]. Stacking often involves a depot steroid, such as nandrolone decanoate together with an orally administered AAS such as methandrostenolone. According to "Anabolics 2002" [29], an anabolic steroid reference manual, the particular combination mentioned will give extremely good results. Another combination recommended by steroid users is stanozolol in the combination with trenbolone acetate.

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Four of all available AAS seemed to be more used than others [30]; testosterone, nandrolone, methandrostenolone and stanozolol. AAS can be bought legally in some parts of the world, whereas in other countries AAS are classified as illegal narcotic substances. In Fig. (**1**) some of the most commonly used AAS are drawn. These compounds are often administered as prodrugs. The rationale behind the alterations of the chemical structure starting from the native testosterone has been a demand for higher potency and selectivity, prolonged action and an improved bioavailability. The simplest approach from the synthetic point of view is to make prodrugs to prolong action and to achieve depot effects. Nandrolone decanoate and the testosterone esters provide typical examples where the hydroxyl groups at C-17 are used as handles for modifications. Nandrolone decanoate remains one of the most popular anabolics in circulation among abusers of AAS in the world. The drug is easily available and information on nandrolone decanoate and a large variety of other AAS can be found in anabolic steroid reference manuals that are popular among AAS users. Concerning nandrolone decanoate, for mentioning one example, it is stated, "The major drawback for competitive purposes is that in many cases nandrolone metabolites will be detectable in a drug screen for up to a year (or more) after use" [29].

Methandrostenolone, also referred to as metandienone ("Russian"), is characterized by the added 17α methyl group that eliminates the potential oxidation of the 17α hydroxyl group. This manipulation results in a better bioavailability and similar operations to improve bioavailability were previously successfully applied in the development of the oral contraceptives. Furthermore, the C-19 methyl group in testosterone is retained in this steroid and an extra double bond has been introduced in the A-ring. A large number of metabolites of methandrostenolone have been identified, [31]. Stanozolol provides a third example of structural modifications that deliver potent AAS. The pyrazole ring linked to the A-ring, creating a five-ring system, is the characteristic feature of this molecule. Thus, testosterone, nandrolone, methandrostenolone and stanozolol, differ considerably from a chemical point of view. Nandrolone decanoate is considered to be the prototype AAS and is very commonly used in animal studies aiming at achieving a better understanding of the biochemical events associated with adverse behaviors have been performed with this steroid.

Besides the desired anabolic effects leading to an increased strength and larger muscle mass [32], there are many adverse effects associated with the use of AAS, especially when administering high doses of the steroids. For example, acne and gynecomastia are commonly seen among AAS users [21, 33, 34]. The latter, being an effect of aromatization of the A-ring of the steroids, delivering compounds with estrogen activity. Although, for example testosterone is aromatized to estrogen not all AAS serve as good aromatase substrates and consequently the aromatization of AAS takes place to various extent as a function of the steroid structure. Interestingly, in order to

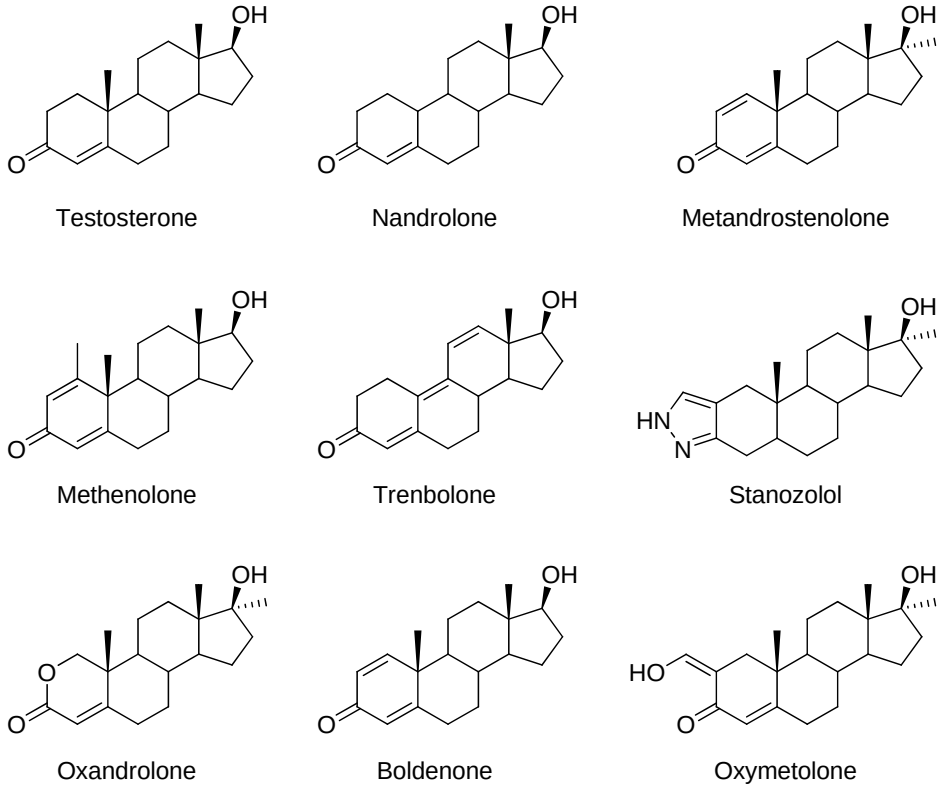

Fig. (1). Examples of some commonly used anabolic androgenic steroids. Some of the steroids are administered orally and others intramuscularly injected as ester prodrugs.

avoid gynecomastia, aromatase inhibitors are also frequently sold on the black-market. Baldness and striae represent other side-effects [34, 35], but also adverse effects such as testicular atrophy, reduction of sperm production and impotence are reported [12]. The effects on testes and sperm production are due to AAS induced suppression of the follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels. The LH and FSH levels, regulating the testosterone production, have been reported to return to normal after withdrawal of the AAS, whereas the concentration of endogenous testosterone remains reduced for a longer period of time [36, 37]. These phenomena are well known among the AAS abusers and thus most of the "recommended" cycle-schedules with steroid stacking end with three weeks of human chorionic gonadotropin administration in order to "kick-start" the endogenous testosterone production.

Administration of AAS affects serum lipoprotein levels, blood coagulation and triglycerides. In addition, fluid retention, hypertension, myocardial infarction, arrhythmia and stroke have been reported in connection to AAS abuse [38-41]. Concerning orally administered 17α-alkylated steroids, such as methyl-testosterone, stanozolol and metandrostenolone, those steroids have been reported to increase the risk for jaundice, hepatic carcinomas and hepatic malignancy [42, 43].

In addition to physical effects, the use of AAS also induces several psychological effects. However, the biochemical mechanisms accounting for the alterations in psychological behaviors are in most cases considerably less understood than those associated with physical effects. Administering high dose of the AAS methyltestosterone has been shown to cause both positive mood such as euphoria, energy and sexual arousal as well as negative mood, including irritability, hostility, violent feelings and mood swings [44, 45]. Furthermore, not only methyltestosterone, but also other AAS, exert similar actions. However, as reported in most studies, a cocktail of different steroids are being used, making it difficult to establish a correlation between certain psychological behaviors and specific steroids.

Suspiciousness, anxiety and irritability are other psychological side effects that have been associated with AAS administration at high doses [46]. Furthermore, aggression and violent behavior are commonly reported in connection to AAS abuse [19, 46-48]. During periods of chronic AAS exposure mania have been observed, while after discontinuation of long-term AAS abuse depression occurred [18, 21, 33, 49, 50].

Several studies have also indicated that AAS might lead to dependence [51-54]. In a study of 100 Australian AAS users approximately 25% met the DSM IV criteria for AAS dependence, as well as for AAS abuse [55]. In another study, 57% of male weight lifters using AAS displayed several symptoms consistent with dependence [56]. In addition to dependence AAS have also been demonstrated to interact with the rewarding pathways for other drugs of abuse. For example, animals pre-treated with AAS have an increased voluntary alcohol intake [57] and also seems to affect the rewarding effects of both cannabinoids and morphine [57-59]. In fact, AAS abuse have been suggested to be a gateway to opiate dependence [60].

EFFECTS OF ANABOLIC ANDROGENIC STEROIDS ON NEUROPEPTIDE SYSTEMS IN THE BRAIN

The biochemical events responsible for the alterations of behaviors that are so frequently observed in connection to AAS abuse are not fully understood and neither are the roles of the various neurochemical systems in the brain. Chronic AAS treatment of rats has been reported to affect both the dopaminergic as well as the serotonergic systems of the brain [61-66]. Furthermore, nandrolone decanoate induces alterations in the gene transcripts of both corticotropin releasing factor (CRF) and proopiomelanocortin (POMC) [67, 68]. AAS also affect the gamma-aminobutyric acid (GABA) [69] as well as the glutamate system [70, 71].

Several adverse behavioral effects, characterizing AAS abusers, could result from alterations in the above-mentioned neurotransmitter systems but also in part be attributed to a disturbance of the delicate balance within neuropeptide systems in the central nervous system. The opioid system, tachykinin system and systems regulating the calcitonin gene-related peptide levels are of special relevance in this context.

The first report on alterations in the endogenous opioid system, as a result of AAS administration, was reported in 1995 [72]. It was found that a cocktail of different AAS had a region specific effect on the β-endorphin immunoreactivity. Two years later it was reported that a remarkable increase in the concentration of β-endorphin in the ventral tegmental area (VTA) was recorded after nandrolone decanoate administration to rats [73]. VTA is one of the brain regions that are assumed to play an important role in the reward system. Furthermore, alterations of opioid peptide levels remained eight weeks after chronic nandrolone decanoate treatment [57]. An AAS induced down-regulation of the delta opioid receptor mRNA in cells has also been reported, a down-regulation that occurs through a mechanism suggested to be independent of the androgen receptor [74].

Considering Met-enkephalin-Arg^{6}Phe^7 (MEAP) and dynorphin B (Dyn B), these two opioids serve as markers of the activity in two genetically different opioid peptide systems. The immunoreactivities of the two peptides in hypothalamus, the major control center in the brain, have been found to be significantly higher after AAS administration [75]. This finding might have implications in the context of AAS abuse since hypothalamus regulates not only autonomic and endocrine responses, but also defensive and aggressive behaviors as well as emotions [76-78]. Significantly higher levels of MEAP and Dyn B were also encountered in the striatum, which is also engaged in controlling emotions, fear and aggression [79]. However, one should bear in mind in this context that the relation between endogenous opioids and aggressive behavior in rodents is somewhat controversial [80]. PAG also displayed significantly higher levels of MEAP and Dyn B after chronic nandrolone decanoate administration. These results further

Table 1. The Amino Acid Sequence of the Discussed Peptides

Peptides	Amino acid sequence
α-CGRP (human)	Ala-Cys*-Asp-Thr-Ala-Thr-Cys*-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser- Arg-Ser-Gly-Gly-Val-Val-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser- Lys-Ala-Phe-NH_2
α-CGRP (rat)	Ser-Cys*-Asn-Thr-Ala-Thr-Cys*-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser- Arg-Ser-Gly-Gly-Val-Val-Lys-Asp-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser- Glu-Ala-Phe-NH_2
Dynorphin A	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln
Dynorphin B (Dyn B)	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr
β-Endorphin (human)	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu
Met-enkephalin	Tyr-Gly-Gly-Phe-Met
Met-enkephalin-Arg^6-Phe^7 (MEAP)	Tyr-Gly-Gly-Phe-Met-Arg-Phe
Substance P (SP)	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
SP_{1-7}	Arg-Pro-Lys-Pro-Gln-Gln-Phe

*indicates a disulfide bond between the Cys^2 and Cys^7 amino acid residues.

support that high doses of AAS affects areas regulating aggressive behavior. In addition, the changes in peptide concentrations induced by the nandrolone administration could probably also affect the sensitive brain reward system. Dynorphin and enkephalin peptides both have regulatory roles with regard to the dopamine transmission in the mesolimbic reward system [81, 82]. Thus, the euphoria and increased self-esteem often observed after AAS use may partly be attributed to stimulation of this system and an increased dopamine activity. Nandrolone decanoate affects the dynorphin and the enkephalin systems not only in striatum, hypothalamus and PAG but chronic administration in addition induced an imbalance in nucleus accumbens [75], a structure reported to be involved in reward mechanisms [83]. Concerning pain perception, opioids are effective as analgesics. While the enkephalins induce their effects mainly *via* activation of DOP receptors, the dynorphins activate mainly the KOP receptors. Through their action on receptors in PAG, the endogenous opioids are able to suppress nociceptive spinal reflexes by acting on the serotonin pathway projecting to the dorsal horn of the spinal cord from nucleus raphe magnum. At the spinal level, substance P release from dorsal horn neurons is inhibited leading to reduced transmission of nociceptive impulses. Hence, the significantly higher levels of MEAP and Dyn B observed in PAG after AAS administration might indirectly suppress substance P release from the dorsal horn neurons and possibly as a consequence and provided that the biochemical alterations can be translated into man, affect the pain perception experienced by AAS abusers.

Substance P (SP), probably the most studied member of the tachykinin family, is the preferred endogenous agonist for the NK1 receptor. Thus, SP exerts its effects as a neurotransmitter and neuromodulator *via* activation of the G-protein coupled NK1 receptor. However, SP effects can also indirectly be achieved through fragmentation of the peptide to its N-terminal bioactive fragment SP_{1-7}. This heptapeptide sometimes exerts similar effects as the mother peptide but also in many cases completely opposite actions from that of SP [84]. Several enzymes, including Substance P endopeptidase (SPE), Neutral endopeptidase (NEP) and Angiotensin converting enzyme (ACE), are capable of generating SP_{1-7} from SP in the CNS [85-87]. Whereas both NEP and ACE are predominantly membrane bound enzymes, SPE, a fairly SP specific enzyme responsible for the formation of both SP_{1-7} and SP_{1-8} has been shown to be present in the CNS in a soluble form.

The AAS nandrolone decanoate has been shown to affect the SP system at several levels, including peptide concentrations, receptor densities and enzymatic processing in important regions of the brain, such as hypothalamus, amygdala, PAG, nucleus accumbens and striatum [88-90]. Thus, chronic treatment with nandrolone decanoate not only has a strong impact on the opioid concentrations but also on the tachykinin levels in brain regions associated with the regulation of emotional behaviors such as aggression, depression and reward.

The NK1 receptor antagonists have been tested as potential antidepressive agents [91, 92]. Although the observed effects in the clinical trials did not fully meet the expectations, this does not necessarily mean that SP might not play an important role in the regulation of depression. It is possible that the depression that is frequently reported as a common side-effect after prolonged usage of AAS is originating from alterations in the SP systems. The higher concentrations of SP observed in the brain of AAS treated rats might be compensated for to some degree by the down-regulation of NK1 receptors. The impact of AAS treatment on the SP/NK1 system in man is not known but one might speculate that symptoms of depression are emerging from long-term NK1 receptor stimulation. In fact, depression is the second most reported adverse effect linked to use of AAS according to the Swedish anti-doping hotline [30].

In the amygdala, an important region for the regulation of affective behaviors, significantly enhanced levels of both SP (28%) and CGRP (77%), although not of the opioids Dyn B and MEAP, were observed after two weeks of chronic nandrolone decanoate administration [75]. After the same period, the concentration of SP_{1-7} tended to be decreased [88]. When comparing the levels of SP and SP_{1-7}, a higher ratio was found between the two peptides in the amygdala, indicating a possible decreased enzymatic activity in this structure. In fact, a tendency towards a lower SPE-like activity in amygdala was also observed in this study. However, suppressed activities of other SP degrading enzymes such as NEP and ACE could also possibly contribute to the higher SP/SP_{1-7} ratio. Both ACE and NEP have been identified in moderate concentrations in amygdala [93, 94]. Furthermore, the density of NK1 receptors in basolateral amygdala was attenuated by 23% after nandrolone administration, an observation that can be attributed to down-regulation / internalization of the NK1 receptor in response to the elevated SP levels. In gerbils, immobilization stress induced a pronounced NK1 internalization in the basolateral amygdala, an effect that was concluded to be due to SP release. This internalization was also reduced by pretreatment with a SP antagonist [95]. Furthermore, SP seems to be released in amygdala in guinea pig pups in response to psychological stress caused by maternal separation [92]. SP has been shown to play a role not only in affective disorders such as depression, as mentioned previously, but also in anxiety. Hence, microinjections of SP into the medial amygdala induce a dose dependent anxiogenic behavior [96]. Amygdala is not only an important region for the regulation of anxiety and depression but also for aggression. In fact, SP neurons originating in the medial amygdala project to the hypothalamus, a brain region where SP release induces defensive rage in cats [97]. Although, there appears to be some differences between the cat and rat with regard to systems involved in mediating aggression, the importance of amygdala and hypothalamus for aggressive responses seems to be similar in both species [98]. Thus, with regard to the nandrolone decanoate induced biochemical alteration observed in amygdala it is tempting to suggest that side-effects such as anxiety, depression and aggression often reported in connection to AAS abuse might partly be due to altered SP and CGRP levels in this region. However, other neural systems such as serotonin, dopamine, GABA and the glutamate systems have important roles.

In hypothalamus, nandrolone administration have been reported to increase the levels of SP, MEAP and Dyn B. However, after a three week treatment-free recovery period the levels were back to normal [75, 88]. Although a lower SPE-like activity was seen in this region after nandrolone treatment [90] no alteration was observed in the concentration of SP_{1-7}. Furthermore, a study showed a decrease in the NK1 receptor density in both the ventromedial hypothalamic nucleus, as well as the dorsal part of the dorsomedial hypothalamic nucleus [89], reflecting a possible decreased biosynthesis or internalization of the NK1 receptor in response to the higher concentrations of SP induced by the AAS treatment. Treatment with AAS can also increase aggression by altering vasopressin activity in anterior hypothalamus [99]. Interestingly, the anterior hypothalamus have been reported to be important region when facilitating AAS-induced aggression through a mechanism involving dopamine and the D2 receptors [100].

Aggression is frequently reported in connection to AAS use and in fact it is the most commonly reported side-effect according to the Swedish anti-doping hotline [30]. Rats chronically treated with nandrolone decanoate display an increased defensive aggression [57] and are in addition more dominant in a competitive situation [66]. Pretreatment with nandrolone decanoate has also been shown to enhance amphetamine-induced aggression [101]. Furthermore, chronic treatment with AAS increased aggression in male adolescent hamsters [99]. Thus, animal models provide good support for the hypothesis that AAS induce aggression. However, in this context it is important to emphasize that not all AAS induce aggression [102]. In fact, the AAS stanozolol seems to suppress aggression [103].

In the PAG, the concentrations of both SP and SP_{1-7} increased after two weeks of chronic nandrolone treatment [88]. The higher level of SP also remained after a three-week recovery period, whereas the increased level of SP_{1-7} was abolished after this period. Interestingly, SP has previously been shown to produce anxiogenic effects in rats when injected into the PAG, whereas the N-terminal fragment SP_{1-7} displayed an opposite action [104]. This anxiolytic effect attributed to SP_{1-7} was however reported not to be mediated by the NK1 receptor. Considering the opposite actions of the two peptides i.e. anxiogenic versus anxiolytic effects and that the SP_{1-7} concentration increased by 40% (SP increased by 23%) [88], and provided that the efficacy of the two peptides were the same, one could speculate that the sum of the two effects would be an anxiolytic outcome after the two-weeks of nandrolone treatment. In fact, rats treated with nandrolone during 14 days have been shown to be less anxious as deduced from studies of fleeing and freezing behavior [57]. On the other hand, only the elevated levels of SP remained and thus one would presume an overall anxiogenic outcome after a recovery period. However, since anxiety has been reported both during ongoing and after AAS abuse in humans, this effect might be individual or species specific. The impact of AAS on SP/SP_{1-7} ratios in human is not known and not only SP, but also those in an anxiety context more well known GABA and serotonergic systems, play central roles in the modulation of anxiety. Notably, it has been reported that AAS in rats can induce significant modulation of GABAergic transmission in rat brain regions essential for endocrine functions [105]. Both stanozolol and 17α-methyltestosterone significantly inhibited the binding of a benzodiazepine site in rat brain [106]. Thus, AAS can directly modulate the $GABA_A$ receptor, for a review see [107].

Whereas SP exerts a nociceptive action in the spinal cord, SP can produce an analgesic effect when injected into the PAG [108]. This could be an indirect effect mediated through SP induced release of enkephalins [109]. Indeed, the concentrations of MEAP, a biomarker for enkephalin activity, were increased after chronic AAS treatment in our experiments. Thus, it is possible that the increased levels of MEAP could be attributed to increased levels of SP in PAG

as a result of nandrolone exposure. Furthermore, the attenuated density of NK1 receptors observed could be a secondary effect of feedback regulation, or possible internalization of the receptor, in response to the increased SP levels observed.

AAS have been reported to have rewarding properties and studies have shown both conditioned place preference when administering testosterone and also oral self-administration to rodents [110, 111]. Interestingly, a study reported that the conditioned place preference could be blocked by administering a dopamine antagonist in the nucleus accumbens in the rat [112], indicating that AAS might have a stimulatory action in the brain reward system. The nucleus accumbens, an important region for the brain reward system, can be subdivided into the nucleus accumbens core and shell. Whereas the shell is coupled to emotion, the nucleus accumbens core is coupled to motor functions [113]. Nandrolone decanoate had a significant impact on the level of SP_{1-7} in nucleus accumbens, increasing the SP_{1-7} content by a remarkable 47% [88]. After a treatment-free recovery period, the increased SP_{1-7} concentration still tended to remain elevated. Since neither SP nor the SPE-like activity was altered in this region after nandrolone administration, it is tempting to suggest that other endopeptidases have been engaged in the generation of SP_{1-7}. Thus, a nandrolone induced biosynthesis of SP, combined with an enhanced enzymatic processing of SP for example by NEP or ACE might account for the high levels of SP_{1-7}. If this would be the case, the net-effect in the nucleus accumbens could be unaltered SP levels and higher concentrations of SP_{1-7}. Furthermore, nandrolone decanoate down-regulated the NK1 receptors in the nucleus accumbens core, but not in the shell [89].

The brain reward system consists of several important brain regions including nucleus accumbens, VTA, frontal cortex and amygdala. Some dopaminergic neurons originating in nucleus accumbens project to the VTA. Thus, one can speculate that the pronounced increase of SP_{1-7} levels might exert an impact on the reward system. When injecting SP or SP fragments into the nucleus accumbens in rats, SP and its C-terminal fragment attenuated passive avoidance behavior while SP_{1-7} was shown to exert the opposite effect, enhancing this behavior [114].

Striatum is a region displaying a high content of SP and a high density of NK1 receptors. Furthermore, the striatum seems to possess a considerable SPE-like activity [90]. Striatum is primarily associated with the motoric system but also with altered behaviors as results of drug abuse [115]. Chronic treatment with nandrolone decanoate increased the levels of SP, Dyn B and MEAP in the striatum, effects that disappeared after the three-week recovery period. Notably, the level of the bioactive SP_{1-7} decreased in the same region and in this case, the level of SP_{1-7} also remained attenuated after the recovery period. In addition to the reduced level of SP_{1-7}, the AAS administration was found to significantly reduce also the SPE-like activity in striatum [90]. Hence, as expected, a low SPE-like activity should provide lower levels of SP_{1-7} and higher levels of SP. Thus, it seems like SPE is an important endopeptidase regulating the balance between SP and SP_{1-7} in striatum.

In a study nandrolone decanoate treated rats displayed a 26% density reduction of NK1 receptors in dentate gyrus [89], an important relay through which cortical projections reach the hippocampal formation. Although no biochemical changes were reported in the hippocampus, it is possible that the SP levels in certain regions of the hippocampus could be altered by nandrolone treatment, although the overall SP concentration in hippocampus remained unaffected. It has also been reported that the number of SP containing neurons in dentate gyrus are lower in patients suffering from Alzheimer's disease [116, 117]. Nandrolone is known to suppress testosterone plasma levels by feedback regulation [118], and men who develop Alzheimer's disease have also been reported to have lower testosterone levels [119]. Thus, although nandrolone activates the same receptor as testosterone and potentially should compensate well for the lower testosterone levels during long-term use of AAS, nandrolone could still have an effect on the biochemical events linked to memory and dementia. In fact, intake of AAS has been reported to be associated with forgetfulness and distractibility [120]. In rats AAS have been reported to impair both the social memory [121] and also under certain conditions the spatial memory [122]. In the latter study the impaired memory was detected using the Morris water maze. Interestingly, no AAS mediated effects were seen when using the radial arm maze to measure spatial memory [123, 124]. The nandrolone-induced effects on social memory were suggested to mediated through the central androgen receptors [121]. However, it is possible that other mechanism also could have an impact on the relation between memory and AAS.

Nandrolone have also been demonstrated to have an impact on the CGRP levels in the anterior pituitary. The levels of CGRP were reduced by 45% after the AAS treatment, an effect that remained after a three-week recovery period [125]. Both SP and CGRP nerve fibers innervate the anterior pituitary in the rat, although at a smaller extent than in other species [126, 127]. The neuropeptide CGRP seems to have a regulatory role in the region and induces ACTH release from anterior pituitary cells from rat [128]. Thus, considering the low levels of CGRP found in this structure [125] it is less surprising that the levels of ACTH were reported to be reduced in power athletes using AAS [36].

It has been demonstrated that SP and CGRP frequently co-exist in the CNS [129]. CGRP has also been reported to act as an inhibitor of SPE [130]. Thus, increased levels of CGRP in regions with SPE-like activity and where SP is present will probably lead to a decreased formation of SP fragments. Since SP_{1-7} is the major metabolite formed from SP by a SPE-like activity it is tempting to suggest that some of the outcome attributed to CGRP in the CNS might as well be effects mediated by increased levels of SP or alternatively be due to a decreased formation of SP_{1-7}. CGRP has further been reported to serve as a signaling molecule that induces expression of NK1 receptors in the CNS [131]. Thus, it seems that increased levels of CGRP can both inhibit the degradation of SP as well as increase the NK1 receptor density.

Although the impact of AAS on neuropeptide systems has been the main focus for this review it should be emphasized that it is known that AAS administration to rats also effect other systems with high relevance for the altered behaviors attributed to AAS abuse. These include AAS impact on e.g. the serotonin, dopamine and glutamate systems [62, 63, 71]. It should also be emphasized that the high doses and accumulated levels of nandrolone could lead to activation also of other related steroid receptors such as estrogen, progesterone and mineralcorticoid, as well as glucocorticoid receptors. Furthermore, activation of membrane bound steroid receptors or neurosteroid receptors, e.g. $GABA_A$ and NMDA receptors, could all contribute to the observed alterations of the neuropeptide systems. AAS or their sulfate conjugates could also interact with neurosteroid receptors or alternatively AAS could indirectly modulate levels of endogenous neurosteroids.

CONCLUSION

The majority of all studies dealing with the impact of AAS on the biochemistry in the CNS have been conducted in animal models. The impact of biological and behavioral data recorded in experimental animal models on the human situation has indeed been widely discussed. In many aspects, it has been questioned whether it is possible to extrapolate observations seen e.g. in the rat to human. Are the effects induced by AAS on brain biochemistry and behavior in male rats in agreement with those the steroids would give rise to when they are injected in young men?

Effects, which are comparable between rats and man would occur in behaviors that results from alterations in brain circuits that are similar in rat and human. On the other hand, it would be difficult to extrapolate changes in behaviors and related chemistry in rat without corresponding behaviors and neurochemistry in human. Regarding primitive functions and behaviors related to the emotional centers in the brain, e.g. the limbic area, there are close similarities between rats and primates. For instance, defensive aggression, reward and factors involved in memory and cognition. On the other hand, factors that account for evaluation, interpretation and conclusion of events that appear in connection with intake of drugs such as AAS may be different. The more extended cortical regions in man compared to rat may include brain circuits and mental capabilities which highly exceed those in rodents and therefore the steroids may affect the human brain in a more sophisticated mode, both with regard to brain chemistry and behavior. Therefore, in attempts to extrapolate data recorded in animals to human it is essential which type of behaviors and which kind of brain circuits the researcher has taken under consideration.

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