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The Buzz About Anabolic Androgenic Steroids: Electrophysiological Effects in Excitable Tissues

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Abstract

Anabolic androgenic steroids (AAS) comprise a large and growing class of synthetic androgens used clinically to promote tissue-building in individuals suffering from genetic disorders, injuries and diseases. Despite these beneficial therapeutic applications, the predominant use of AAS is illicit: these steroids are self-administered to promote athletic performance and body image. Hand in hand with the desired anabolic actions of the AAS are untoward effects on the brain and behavior. While the signaling routes by which the AAS impose both beneficial and harmful actions may be quite diverse, key endpoints are likely to include ligand-gated and voltage-dependent ion channels that govern the activity of electrically excitable tissues. Here we review the known effects of AAS on molecular targets that play critical roles in controlling electrical activity, with a specific focus on the effects of AAS on neurotransmission mediated by $GABA_A$ receptors in the central nervous system (CNS).

Keywords

Anabolic steroid; androgen receptor; $GABA_A$ receptor; allosteric modulator; potassium channel; forebrain; aromatase

Anabolic Androgenic Steroids

Anabolic androgenic steroids (AAS) comprise a large class of synthetic androgens originally designed to treat hypogonadism in men and developmental endocrine disorders (1, 2) that are currently also used in the treatment of a wide range of wasting syndromes (3). While still valuable for the treatment of disease, the non-therapeutic tissue-building benefits of AAS were quickly recognized outside of the clinic. Over the past several decades, the predominant use of AAS has become illicit, with individuals of both sexes administering these drugs to enhance athletic performance or body image (4–7). Adult men are reported to self-administer AAS at concentrations that reflect 10–100x therapeutic doses of testosterone prescribed to restore circulating levels of this gonadal androgen in hypogonadal men (6–11). Girls and women are reported to take AAS at levels equivalent to or even exceeding those administered by men (5, 8, 12). Thus the same doses self-administered by men may be expected to yield circulating levels of androgens in women and girls, both synthetic and physiological, that are orders of magnitude higher than their normal physiological levels of androgens (8).

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Illicit steroid use is typified by elaborate and concurrent combinations of multiple AAS (stacking) with waxing and waning concentrations (pyramiding); regimes that are believed by the users to limit production of catabolic molecules, such as cortisol, and to minimize untoward side effects (5, 13). Steroid users also typically co-administer drugs such as 5α-reductase inhibitors (finasteride) to minimize untoward effects of steroids, as well as other non-steroidal compounds such as insulin for energy replacement (insulin) and triiodothyronine for fat loss (5, 13). The number of individual AAS that have been synthesized is large and continuing to grow, particularly with the development of designer steroids that may be packaged as unregulated ingredients in nutritional supplements (14). Despite this diversity, all AAS are synthetic derivatives of testosterone that can be broadly grouped into three classes. The first class of AAS includes compounds derived from esterification of the 17β-hydroxyl group of testosterone that are commonly injected and are represented by testosterone propionate and testosterone cypionate. Testosterone esters can be hydrolyzed into free testosterone, reduced to 5α-dihydrotestosterone (DHT), an androgen with higher biological activity at the androgen receptor (AR) than testosterone (4, 15, 16), or aromatized to estrogens (4, 15). Molecules that have been 5α-reduced cannot be metabolized into estrogens, but may be metabolized into other androgens, such as 3α-androstanediol (4, 15). The length of the ester moiety determines both the half-life and the purported desirability of the steroid as an anabolic drug (http://www.steroid.com/Testosterone-Propionate.php). The second class of AAS includes the 19-nortestosterone derivatives. These compounds have a hydrogen atom substituted for the methyl group of testosterone at C19. The 19-nortestosterone derivatives can also be injected and are typified by nandrolone or its ester, nandrolone decanoate (1, 2). Like the testosterone esters in the first class, AAS in this second class can be aromatized to 17β-estradiol, although not as well as testosterone (15, 17), accounting for their popularity among illicit steroid users (http://www.steroid.com/Deca-Durabolin.php). The third class includes compounds such as methandrostenolone that are alkylated at C17. Because alkylation retards metabolism by the liver, this group of AAS is orally active (1) and is often used for quick gains between cycles (http://www.steroid.com/Dianabol.php). None of the 17α-alkylated steroids is converted into DHT or aromatized to 17β-estradiol (15, 17, 18), although they may be converted to other androgenic and estrogenic metabolites (19, 20).

Despite the explosion in the number of different AAS that have been synthesized, all AAS are active at the AR and thus have androgenic, as well as anabolic, properties (21–23), although AAS user websites may claim otherwise. In addition to AR-mediated actions, the AAS can also impose important biological actions via estrogen receptor alpha (ERα) and ERβ, either directly (24) or following aromatization (25–28), as well as directly via progestin receptors (29). Beyond their interactions with these classical nuclear hormone signaling pathways, the AAS can elicit rapid effects through interactions with a non-AR/ER microsomal binding site (30), by allosteric regulation of enzymes involved in the biotransformation of steroids (20, 27, 31, 32), and by allosteric modulation of ion channels (33). Thus, despite recent commentary to the contrary (34), the chemical modifications imposed to alter the properties of the AAS, as well as the concentrations at which they are present in steroid users, have endowed them with abilities to signal via mechanisms that endogenous androgens under normal physiological conditions do not. In order to understand the full repertoire of the consequences of AAS use, one therefore must certainly include, but also move beyond, the realm of classical nuclear AR signaling and consider distinct actions these synthetic steroids have via alternative pathways. The best studied of these non-classical actions of AAS, especially with respect to their effects in the CNS, is their role as allosteric modulators of the $GABA_A$ receptor.

$GABA_A$ Receptors

Structure of the $GABA_A$ Receptor

The $GABA_A$ receptor is a widely expressed anion-selective ligand-gated channel that subserves an essential role in mediating fast inhibitory transmission in the CNS. The native receptor is a pentameric transmembrane protein for which sixteen different receptor subunit genes (α_1-α_6, β_1-β_3, γ_1-γ_3, δ, ε, π, and θ) have been identified in mammals. Subunit composition determines the basic biophysical properties of the receptor, such as the conductance, mean open time, and the rate of desensitization, and the ability of a wide range of substances to allosterically modulate channel function. Expression of different subunit genes, and their incorporation into functional receptors shows marked region-specific and developmental variation and is governed by changes in the levels of numerous different endogenous regulators, as well as by exogenous drugs (35–37). The AAS have both acute and chronic actions on the $GABA_A$ receptor. They can impose immediate and significant changes in channel function through direct allosteric modulation of the receptor. They also alter $GABA_A$ receptor-mediated synaptic transmission through long-term changes in subunit mRNA expression and resultant changes in channel function and through affecting the presynaptic release of GABA.

Allosteric Modulation of $GABA_A$ Receptor Function by Acute Actions of AAS

The 1990's saw the first studies to suggest that the AAS have significant effects on brain function through their actions at the $GABA_A$ receptor (38, 39). Specifically, prolonged exposure to supraphysiological concentrations of testosterone propionate or methandrostenolone was found to left-shift the EC_{50} for chloride flux induced by GABA in rat cortical synaptosomes, leading the authors to propose a direct action on the $GABA_A$ receptor. While the findings were intriguing, the investigators proposed that the allosteric actions were not due to the AAS themselves, but that the effects arose secondarily following metabolism of the AAS to neurosteroid derivatives; agents already known to allosterically modulate the $GABA_A$ receptor (40). However, the prolonged nature of the treatment, the lack of correlation of behavioral (anxiolytic effects) and serum neurosteroid levels, and the lack of data indicating that methandrostenolone (a 17α-alkylated AAS) could be metabolized to a neurosteroid, begged an alternative explanation. Demonstration that the AAS can directly impose allosteric modulation on the $GABA_A$ receptor was soon to follow (41, 42). In these studies, the investigators not only demonstrated enhancement of chloride flux by two 17α-alkylated AAS, but also provided important pharmacological data to indicate distinct functional mechanisms and separate binding sites for the AAS and the endogenous neurosteroids. Direct electrophysiological data of acute allosteric modulation of $GABA_A$ receptor function by 17α-alkylated AAS came with the advent of the technique of ultrafast perfusion (43–45). These studies provided further evidence that the AAS differ from both gonadal steroids and endogenous neurosteroids since 17α-methyltestosterone (17α-MeT), but not testosterone, was shown to be able to elicit allosteric modulation of the $GABA_A$ receptor; that 17α-MeT at concentrations as high as 10 μM did not directly gate the channel (as can the neurosteroids; (46)); and that the mechanisms by which this AAS altered channel gating are distinct from those for the endogenous neurosteroids (37). Specifically, for receptors that do not contain the δ subunit, positive neurosteroids show little dependence on subunit composition (46) and act to slow the rate of recovery from desensitization and prolong deactivation (47, 48). For δ-containing receptors, positive neurosteroids elicit dramatic enhancement of currents by stabilizing the open state of the channel (49). In contrast to the positive neurotsteroids, AAS modulation of currents depends upon α (43, 44), as well as δ (44) and ε (45) subunit composition of the receptor. Specifically, 17α-MeT, imposes dramatically different actions at α_1- versus α_2-containing ($\alpha_x\beta_3\gamma_2$) receptors and modulates gating through distinct kinetic mechanisms: This AAS promotes entry into

singly-liganded open states for α_1-containing receptors (43), but preferentially enhances the stability of more distal open and desensitized states while destabilizing the proximal desensitized state for α_2-containing receptors (44). While positive neurosteroids dramatically enhance currents through δ-containing receptors, AAS are without effect (44). For ε-containing receptors, 17α-MeT actually inhibits both the ligand-gated and spontaneously open states of the channel (45, 50) through an allosteric block in which this AAS interacts preferentially with and promotes accumulation in a closed state (45). The disparity in functional effects of the AAS versus the neurosteroids is consistent with the divergence in structural signatures of the two classes of molecules. In particular, neither the 3α-hydroxyl moiety common to all active neurosteroids nor the C_{17} or C_{20} keto group (51) is present in any of the AAS (52).

The mechanism by which 17α-MeT modulates the receptor is also distinct from that of another well-known class of allosteric modulators, the benzodiazepines, acting at their high affinity binding site (43, 44). Moreover, in contrast to the benzodiazepines, modulation elicited by 17α-MeT is independent of γ subunit composition (36). Taken together, these studies indicate that in addition to their long-term actions mediated by nuclear hormone receptors, the AAS may have significant effects on neural processing via acute alterations in $GABA_A$ receptor function that have similarities to, but are nonetheless distinct from, other important psychoactive modulators of this channel.

Changes in $GABA_A$ Receptor-Mediated Transmission Elicited by Chronic Exposure to AAS

i. Neural regions that regulate sexual behaviors and reproduction—

Neurotransmission mediated by $GABA_A$ receptors regulates pubertal onset, reproductive function, and sexual behaviors (52–56). One of the seminal outcomes of chronic use of high concentrations of AAS is disruption of these functions (52, 53). In human subjects, AAS use is not limited to adults, and 2–5% of high school students are estimated to illicitly administer AAS (57, 58). Early AAS use in teenagers (7, 57) engenders heightened concern since risks associated with its use may be greater in adolescents than in adults, given the greater hormone sensitivity of the brain during this period (59, 60).

Chronic exposure during adolescence to the AAS, 17α-MeT, disrupts reproductive function in both male and female mice (52, 53). In both sexes, interference with peripheral reproductive state was found to be accompanied by significant decreases in the frequency of action potentials in gonadotropin releasing hormone (GnRH) neurons (61, 62); neurons that mediate the final control over the hypothalamic/pituitary/gonadal (HPG) axis (56). AAS treatment had no direct effect on the expression of $GABA_A$ receptor subunit mRNAs in identified GnRH neurons, nor did treatment alter the amplitude or decay kinetics of $GABA_A$ receptor-mediated spontaneous postsynaptic currents (sPSCs), miniature sPSCs or tonic currents in these cells. These data indicate that AAS treatment was without an appreciable effect on the complement or function of postsynaptic $GABA_A$ receptors expressed in GnRH neurons (61, 62). However, treatment did result in a significant increase in the frequency of sPSCs onto GnRH neurons in both sexes, consistent with a significant effect of AAS exposure on neurons that provide afferent GABAergic innervation to the GnRH cells. In male mice, this increase in sPSC frequency could be attributed to augmented activity of presynaptic GABAergic neurons within the medial preoptic area (mPOA) (61). While studies in female mice also demonstrated significant effects of chronic exposure to 17α-MeT on the activity of neurons within the mPOA, the actions were not correlated with concomitant changes in GnRH neuronal activity. Rather data from this study suggested that AAS impart their actions on GnRH neuronal function by altering the activity of afferent kisspeptin-expressing neurons in the anteroventral periventricular nucleus (AVPV) (62). The identification of upstream neurons in the mPOA and the AVPV as the targets of long-term AAS effects is consistent with the marked level of expression of both AR and ERα/β in

these afferent regions (63–65), and the dearth of AR and ERα in the GnRH neurons themselves (66–68) (Figure 1A, B).

While studies examining the effects the AAS, 17α-MeT, have provided important mechanistic information about the actions of the AAS, exposure to only one single compound does not reflect typical patterns of steroid usage in human subjects (5). Studies of adult male and female mice treated with a mixture of commonly abused AAS (testosterone cypionate, methandrostenolone and nandrolone decanoate) more likely to reflect a regime administered by people, have also revealed significant effects of chronic AAS treatment on neurons within the mPOA. Moreover, these studies demonstrated that AR signaling is critical in mediating some, but not all of these effects, and, as noted above, that sex-specific differences were evident in the actions of this mixture of AAS on specific neuronal populations. In adult male mice, chronic treatment with this AAS mixture promoted enhanced action potential firing of neurons within the mPOA (27), while this same treatment led to significantly lower levels of activity in mPOA neurons of female mice (69). In both sexes, prolonged exposure to this mixture led to significant increases in the expression of the α_5 subunit mRNA (27, 65) and in changes in $GABA_A$ receptor-mediated synaptic currents that were consistent with augmented expression of α_5-containing receptors (65). Loss of AR-mediated signaling, either pharmacologically by concomitant treatment with the AR antagonist, flutamide, (69) or genetically by germ line loss of functional AR in the testicular feminization (*Tfm*) mutant mouse (27), abrogated the AAS-induced effects on action potential firing and α_5 expression.

Interestingly, significant actions of chronic AAS were nonetheless evident in the *Tfm* mice, indicating that physiological actions of these synthetic steroids can be mediated by AR-independent means (27). Specifically, AAS treatment of *Tfm* mice elicited a significant decrease in the frequency and amplitude of $GABA_A$ receptor-mediated spontaneous inhibitory postsynaptic currents (sIPSCs) and a significant decrease in levels of the mRNA encoding the 65 kDa isoform of the GABA synthesizing enzyme, glutamate decarboxylase, in the mPOA. Experiments in this study went on to show that the electrophysiological effects on $GABA_A$ receptor-mediated currents could be attributed to AAS-dependent inhibition of aromatase activity and thus antagonism of endogenous ER-mediated actions that normally augment GABAergic tone in the mPOA (70). These data in the mammalian CNS (27) are consistent with previous studies in non-neuronal cell lines (20, 31) and in non-mammalian vertebrates (32) demonstrating the ability of the AAS to inhibit the activity of aromatase.

ii. Neural regions that regulate anxiety, fear, and stress—In addition to effects on reproduction, chronic AAS use in people is associated with a plethora of effects on affect, including depression, mania, hypomania, somatization, increased anxiety, irritability, extreme mood swings, abnormal levels of aggression, body dysmorphia and paranoia (6, 10, 71–74). Recent studies in mice provide information on fundamental mechanisms that may underlie some of these actions in demonstrating that chronic AAS treatment alters GABAergic transmission in neural circuits that are critical for the expression of fear, anxiety and depression. Specifically, treatment of female mice during adolescence with a mixture of AAS (methandrostenolone, nandrolone decanoate and testosterone cypionate) significantly augmented firing of neurons from the central amygdala (CeA) that project to the bed nucleus of the stria terminalis (BnST) and $GABA_A$ receptor-mediated inhibition in these target BnST neurons (75). This projection provides an essential limb of the neural circuitry within the extended amygdala that is crucial for the generation of generalized anxiety (76). Consistent with altered transmission in this pathway, AAS treatment increased anxiety-like behavior as determined by the acoustic startle response and the elevated plus maze (75, 77). As with the effects of 17α-MeT on GABAergic afferents to GnRH neurons (61, 62), the

observed effects of chronic exposure of this AAS mixture at the CeA to BnST synapse were presynaptic: The treatment promoted an increase in $GABA_A$ receptor-mediated sIPSC frequency, but no change in the amplitude or kinetics of either sIPSCs or mIPSCs in the BnST neurons (75). The ability of the AAS to elicit both the changes in anxiety and the augmentation of GABAergic inhibition in the BnST were dependent on corticotropin releasing factor (CRF) signaling at the type 1 receptor (75, 77). While the direct role of AR, ER or other nuclear hormone signaling pathways was not tested in this study, acute exposure to this AAS mixture did not elicit anxiogenic behaviors. Moreover, acute exposure to the steroid mixture had only postsynaptic (allosteric) effects on $GABA_A$ receptor-mediated sIPSC amplitudes; no effect on frequency (75). These data suggest that AAS actions through nuclear hormone signaling pathways are likely necessary to mediate the effects on GABAergic transmission at the CeA to BnST synapse (Figure 1C). It is also interesting to note that the actions of AAS in promoting a CRF-dependent increase in the release of GABA onto BnST neurons are highly reminiscent of the effects of chronic ethanol exposure on GABAergic afferents to the CeA neurons themselves (78–80). Data determining the actions of ethanol on GABAergic transmission in these neurons highlight intriguing molecular avenues, such as the role of nociceptin/orphanin FQ (81), that should be explored with regard to mechanisms by which the AAS may lead not only to augmented GABA release, but also possibly changes in glutamatergic transmission in the extended amygdala (82).

In addition to augmenting presynaptic release of GABA via this CRF-dependent mechanism, recent studies have also illuminated a separate critical mechanism by which chronic AAS treatment may alter GABAergic transmission in neural circuits important in fear, anxiety and depression. Specifically, it has been shown that chronic exposure to even relatively low doses of AAS inhibits the production of 5α-reductase type I, and thus, the bioavailability of pregnane steroids, such as allopregnanolone, via an AR-mediated mechanism (26, 83–85). While the electrophysiological correlates of AAS-mediated decreases in the concentrations of positive neurosteroid modulators were not examined in these studies, the most straightforward expectation would be for diminished GABAergic inhibition within neural circuits that express appreciable levels of 5α-reductase type I and are implicated in the expression of fear and anxiety. Such expectations are consistent with data indicating that both increased anxiety (76) and contextual fear conditioning (86) involve decreases in GABAergic inhibition and disinhibition of key neural elements in these circuits.

Although AAS treatment of adult female mice was found to decrease the level of 5α-reductase mRNA expression in glutamatergic neurons of the basolateral amygdala by 70%, no changes were observed in the levels of mRNA for this enzyme in neurons of the central amygdala. Moreover, AAS treatment did not alter the levels of the transcript for this enzyme in GABAergic output neurons of the thalamus [85]. Such region- and cell-specific effects of AAS treatment are consistent with the observation that AAS treatment did not lead to significant changes in the time course of $GABA_A$ receptor-mediated postsynaptic current decay in BnST neurons (75); a result that may reflect low levels of expression of 5α-reductase in the CeA projection neurons, and thus a lack of significant AAS-induced effect on neurosteroid modulation of postsynaptic GABAergic currents in the BnST.

An intriguing corollary to emerge from studies of the effect of the AAS on ion channels in the CNS is the observation that both social isolation (84) and chronic AAS exposure (27, 69) increase the expression of the α_5 subunit of the $GABA_A$ receptor in regions of the forebrain involved in the production of anxiety and aggression (87) (including the medial preoptic area (27, 69, 88), the amygdala (88), the hippocampus (84), and the frontal cortex (84, 89)) and enhance aggression and anxiety like-behaviors (26, 75, 77, 90). As GABAergic

transmission mediated by α_5-containing receptors has been shown to be important in trace fear conditioning (91–93) and α_5 expression is altered in high anxiety versus to normal lines of mice (94), these data suggest that changes in forebrain neurotransmission mediated by α_5-containing receptors could play a significant role in AAS-induced fear, aggression, and anxiety.

It is also interesting to speculate that potential actions of the AAS, especially as they relate to fear and anxiety, may involve effects on GABAergic transmission that arise due to changes in the expression of neurotrophins and/or their receptors. Chronic treatment of adult male rats with either nandrolone decanoate or stanozolol was shown to result in significantly lower levels of brain-derived neurotrophic factor (BDNF) in the hippocampus and prefrontal cortex and to increase depression-like behavior (95); cf (96). BDNF can act to inhibit GABA receptor-mediated transmission (97) and may cause rapid sequestration of $GABA_A$ receptors in cultures of hippocampal or amygdalar neurons (98). In addition, repeated cocaine exposure has been shown to result in elevated levels of BDNF in medial prefrontal cortex, which in turn, led to enhanced long-term potentiation by reducing surface expression of $GABA_A$ receptors and GABAergic inhibition. Thus, chronic abuse of AAS may also alter the balance of inhibition and excitation in CNS circuits that underlie anxiety and aggression by altering the levels of BDNF or other neurotrophins that promote sequestration of $GABA_A$ receptors in these cells.

Finally, it is of great interest to consider the interplay between the actions of the AAS on CRF-mediated increases in inhibition observed in the BnST (75, 77) and to extrapolate these data in thinking about a potential role for CRF as being a key player in imposing AAS-dependent changes in GABAergic transmission to GnRH neurons. The ability of stress, and, in particular CRF, to alter the onset of puberty and suppress reproductive function is a cornerstone of the interactions between the HPG and the hypothalamic/pituitary/adrenal (HPA) axes (99–101). Consistent with the known physiological actions of CRF and GABA on the control of reproduction, CRF mRNA is highly expressed within neurons of the mPOA (102, 103), and stress augments the activity of GABAergic neurons in this region (104). CRF-containing terminals make direct contacts with GnRH neurons (102), and CRF receptor type 1 is expressed in these cells (105). It is intriguing to speculate that AAS-dependent changes in CRF modulation of GABAergic transmission that are comparable to those observed in the BnST may also occur in the mPOA, and that this CRF-dependent modulation plays a pivotal role in the observed changes in the activity of mPOA (61) and AVPV (62) neurons that contribute to AAS suppression of the activity of GnRH neurons and to the negative effects of these steroids on reproductive competence.

Voltage-Gated Ion Channels

To date, no studies have assessed a direct action, either allosteric or genomic, on the function of voltage-gated ion channels within the CNS. However, a handful of pivotal studies on peripheral tissue have demonstrated that AAS can also alter the function of these fundamental molecules of electrical excitability. Chronic exposure to 17α-MeT was reported to cause broadening of the electric organ discharge (EOD) (106). The EOD is a signal that is crucial for navigation, prey capture and species-specific communication in weakly electric teleost fishes which reflects the activity of single electrocytes (modified skeletal muscle-derived cells), as well as pacemaker neurons and their neuronal targets (107). The basis for the broadening of the waveform has been attributed to an AR-mediated slowing of the rate of inactivation of voltage-dependent sodium channels and concomitant slowing of voltage-dependent potassium channel activation (108, 109). Chronic AAS treatment has also been shown to alter the current densities and kinetics of potassium currents (101) and autonomic control of contractility (110) in cardiac muscle and

intracellular calcium homeostasis and the voltage-dependence of potassium contractures in skeletal muscle (111, 112). Finally, chronic AAS treatment has been indirectly shown to alter electrical activity in the CNS as demonstrated by increased staining for the immediate early gene, c-FOS (113–115). However, whether AAS-dependent modulation of Fos reflects changes in synaptic inputs or in voltage-gated conductances and the molecular identity of the channels altered remains to be determined.

Future Directions

At the protein and mRNA levels, chronic AAS exposure has been shown to impart significant effects on the expression or post-translational modification of a host of critical neuronal signaling components, including NMDA receptors (116), serotonin receptors (117–121) and dopamine receptors (122–125). However, no studies to date have explored the electrophysiological consequences of alterations in these receptors as a result of AAS exposure. Similarly, while chronic AAS treatment has been shown to alter sodium and potassium channel function in electrically excitable tissue in the periphery, as noted above no study to date has examined the effects of AAS on the expression and/or function of voltage-gated channels in the CNS. Given the broad range of actions that endogenous androgens have on ion channel function in electrically excitable tissue (33), it is likely that these synthetic steroids also alter neuronal function via effects on voltage-gated channels and intracellular ion homeostasis in the CNS. Assessment of how AAS modify expression of these other receptors and channels and how they influence their function via phosphorylation (96, 126) or direct allosteric modulation remain critical questions that need to be addressed to fully understand the neural and behavioral consequences of both licit and illicit use of AAS.

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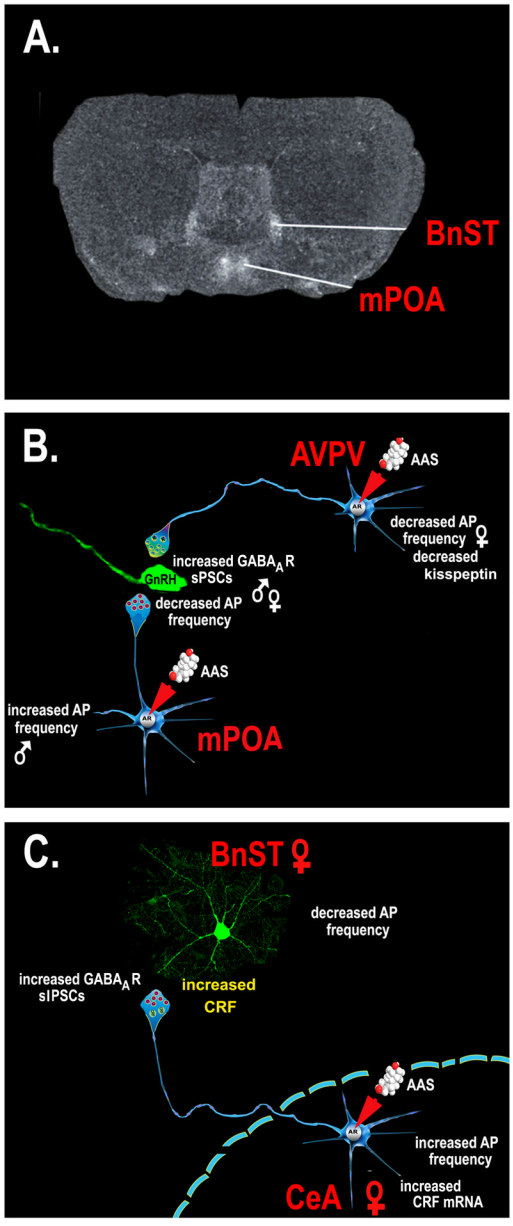

Figure 1. Electrophysiological actions of the AAS on GABAergic transmission in regions of the mammalian forebrain

A. Coronal section of a rat brain demonstrating labeling associated with an antisense mRNA to the γ_1 subunit of the $GABA_A$ receptor. The γ_1 is preferentially expressed in the mPOA and the BnST, and thus provides demarcation of these two regions. B. The AAS, 17α-MeT, acting through the AR promotes an increase in the frequency of $GABA_A$ receptor-mediated spontaneous postsynaptic potentials (sPSCs) and decreased action potential (AP) firing in GnRH neurons of both the male and female mouse. In males, the AAS effects on GnRH neurons can be attributed to an increase in firing in neighboring GABAergic (neurons in the mPOA (61). In females, the decrease in firing of GnRH neurons does not correlate with changes in firing in mPOA neurons, but does correlate with AAS-induced changes in the firing and expression of kisspeptin in neighboring neurons in the AVPV (62). C. In the BnST, treatment of female mice with a mixture of AAS results in an increase in firing of CeA neurons and a concomitant increase in the frequency of $GABA_A$ receptor-mediated

spontaneous inhibitory postsynaptic potentials (sIPSCs) and diminished firing in postsynaptic BnST neurons. The actions of the AAS require CRF signaling through the CRF type 1 receptor (75, 77).