Repeated Anabolic-Androgenic Steroid Treatment during Adolescence Increases Vasopressin V$_{1A}$ Receptor Binding in Syrian Hamsters: Correlation with Offensive Aggression

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Repeated anabolic-androgenic steroid treatment during adolescence increases hypothalamic vasopressin and facilitates offensive aggression in male Syrian hamsters (*Mesocricetus auratus*). The current study investigated whether anabolic-androgenic steroid exposure during this developmental period influenced vasopressin V$_{1A}$ receptor binding activity in the hypothalamus and several other brain areas implicated in aggressive behavior in hamsters. To test this, adolescent male hamsters were administered anabolic steroids or sesame oil throughout adolescence, tested for offensive aggression, and examined for differences in vasopressin V$_{1A}$ receptor binding using *in situ* autoradiography. When compared with control animals, aggressive, adolescent anabolic steroid-treated hamsters showed significant increases (20–200%) in the intensity of vasopressin V$_{1A}$ receptor labeling in several aggression areas, including the ventrolateral hypothalamus, bed nucleus of the stria terminalis, and lateral septum. However, no significant differences in vasopressin V$_{1A}$ receptor labeling were found in other brain regions implicated in aggressive responding, most notably the lateral zone from the medial preoptic area to anterior hypothalamus and the corticomedial amygdala. These data suggest that adolescent anabolic steroid exposure may facilitate offensive aggression by increasing vasopressin V$_{1A}$ receptor binding in several key areas of the hamster brain. © 2002 Elsevier Science (USA)

Key Words: vasopressin V$_{1A}$ receptor; anabolic-androgenic steroids; aggression; vasopressin.

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INTRODUCTION

Studies from the National Institute on Drug Abuse estimate that more than a half million 8th and 10th grade students are using anabolic androgenic steroids (AAS) in the United States each year (NIDACapsules, 2001). Of particular interest are reports that AAS use has risen significantly in this adolescent population, with 3.6% of male 10th graders reporting use in 2000, up from 2.8% in 1999, 2.0% in 1997, and 1.8% in 1996 (NIDACapsules, 2001). This pattern of abuse is important since the onset of AAS use during adolescence (15 years of age or younger) is correlated with more frequent and heavier use later in life (Buckley, Yesalis, Friedl, Anderson, Streit, and Wright, 1988), despite physical or psychological ramifications (Yesalis, 1988). Thus, this population of adolescent users may constitute a significant portion of the stable long-term AAS-abusing population.

Clinical studies have demonstrated a positive correlation between the long-term use of AAS and negative behavioral effects, including increased aggressive behavior (Pope and Katz, 1994; Strauss, Wright, and Finerman, 1983; Su, Pagliaro, Schmidt, Pickar, Wolkowitz, and Rubinow, 1993). However, whether AAS exposure can elicit similar behavioral changes in adolescent animal models has only recently been studied. Behavioral data from our laboratory indicate that Syrian hamsters treated with AAS during adolescent development display elevated offensive aggression (Harrison, Connor, Nowak, Nash, and Melloni, 2000; Melloni, Connor, Hang, Harrison, and Ferris, 1997; Melloni and Ferris, 1996). In these studies hamsters treated with AAS were six to eight times more likely

and twice as fast to attack and bite intruders placed in their home cage. The finding that AAS-treated hamsters displayed heightened offensive aggression on the first behavioral interaction, in the absence of established social interactions and cues, suggested that AAS exposure during this developmental period may stimulate aggression directly, perhaps by impacting the activity of specific neurotransmitters that regulate this behavior.

Arginine vasopressin (AVP) has been implicated in the modulation of aggression in various species and models of aggression, and in humans (Coccaro, Kavousi, Berman, Cooper, Hauger, and Ferris, 1995; Ferris, Axelson, Martin, and Roberge, 1989; Ferris, Delville, Grzonka, Luber-Narod, and Insel, 1993; Koolhaas, Moor, Hiemstra, and Bohus, 1991; Koolhaas, Van den Brink, Roozendal, and Boorsma, 1990). In adult Syrian hamsters, AVP activity in the lateral aspects of the medial preoptic area to anterior hypothalamus (MPOA-AH) and ventrolateral hypothalamus (VLH) has been shown to regulate offensive aggression (Delville, DeVries, and Ferris, 2000; Delville, Mansour, and Ferris, 1996a; Ferris, Melloni, Koppel, Perry, Fuller, and Delville, 1997), where AVP acts to facilitate aggression through an interaction with the AVP V_{1A} subtype receptor (Delville, Mansour, and Ferris, 1996b; Ferris and Potegal, 1988; Potegal and Ferris, 1989). AVP V_{1A} receptor binding is testosterone-sensitive in the MPOA-AH (Johnson, Barberis, and Albers, 1995) and VLH (Delville *et al.*, 1996b). In the VLH there are sex differences in AVP V_{1A} receptor binding, with males showing a higher density of VLH-AVP V_{1A} receptor binding (Delville and Ferris, 1995). However, while AVP V_{1A} receptor binding is sexually different, both males and females exhibit equally high levels of binding density following testosterone treatment (Delville and Ferris, 1995). Moreover, previous studies have shown that testosterone facilitates offensive aggression by modulating AVP V_{1A} receptors within the VLH (Delville *et al.*, 1996b). Together, these results suggest that the MPOA-AH and VLH contain distinct subsets of AVP V_{1A} receptor-containing neurons that are highly responsive to circulating androgens, and that testosterone modulates aggression at least partly through an interaction with AVP V_{1A} receptor within both these brain regions. Perhaps AAS exposure during adolescent development facilitates offensive aggression by a similar mechanism, i.e., by influencing AVP V_{1A} receptor binding activity in the MPOA-AH and VLH, thus potentiating AVP's excitatory influence on aggression. To date, however, it remains un-

known whether adolescent AAS exposure influences AVP V_{1A} receptor binding in these brain regions.

AVP V_{1A} receptors exist in other brain regions implicated in offensive aggression in hamsters, namely, the bed nucleus of the stria terminalis (BNST), lateral septum (LS), and medial nucleus of the amygdala (MeA) (Johnson *et al.*, 1995; Young, Wang, Cooper, and Albers, 2000). Neurons in the BNST (Delville *et al.*, 2000; Kollack-Walker and Newman, 1997) and MeA (Delville *et al.*, 2000), including the corticomedial amygdala (CoMeA) (Potegal, Ferris, Hebert, Meyerhoff, and Skaredoff, 1996) are activated during an aggressive encounter with other hamsters. AVP activity in the LS of hamsters stimulates intense flank marking (Ferris, Delville, Irvin, and Potegal, 1994; Irvin, Szot, Dorsa, Potegal, and Ferris, 1990), a stereotypic motor behavior controlled by AVP that is part of the response pattern of offensive aggression in hamsters (Ferris, Albers, Wesolowski, Goldman, and Luman, 1984). It is possible that adolescent AAS exposure increases AVP V_{1A} receptor activity in these aggression areas as well, thereby stimulating offensive aggression. To date, however, it is unknown whether AAS exposure during adolescence has any effects on the binding of AVP V_{1A} receptors in these brain regions. This information is important given the continued reports that AAS use during this developmental window is rising (NIDACapsules, 2001) and can be associated with an increased incidence of aggression and violence (Nelson, 1989; Pope and Katz, 1988; Pope, Katz, and Champoux, 1988; Strauss *et al.*, 1983).

The present studies were conducted to establish a relationship between adolescent AAS exposure, offensive aggression, and AVP V_{1A} receptor binding activity in areas of the hamster brain implicated in the aggressive response. To this end, we used the subadult Syrian hamster to determine whether adolescent AAS exposure facilitates offensive aggression correlated with enhanced AVP V_{1A} receptor binding in these key brain regions.

METHODS

Animals and Treatment

All animal work was carried out in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* (DHEW Publication 80-23, Revised 1985). Intact adolescent male hamsters (P25) were obtained from Harlan Sprague–Dawley Labs (Indianapolis, IN), individually housed in Plexiglas

cages, and maintained at ambient temperature on a reverse light:dark cycle (14L:10D; lights on at 1900 h). Food and water were provided *ad libitum*. On P27, hamsters were weighed and randomly distributed into three groups. Group one (G1 = AAS-treated, *n* = 6) animals received daily subcutaneous (sc) injections of a cocktail of AAS consisting of 2 mg/kg testosterone cypionate, 2 mg/kg nandrolone deconate, and 1 mg/kg boldenone undecylenate (Sigma Chemical Co., St. Louis, MO) suspended in sesame oil (SO) for 30 consecutive days (P27–56). This treatment regime was designed to mimic a chronic "heavy use" regimen (Pope and Katz, 1988; 1994). As a control, a second group of hamsters (G2 = vehicle, *n* = 6) were injected with SO alone. In addition, a third group of stimulus hamsters (G3 = intruders, *n* = 15) was used solely to test the aggressive behavior of G1 and G2 animals. On the day following the last injection (P56), animals from each group (G1 and G2) were tested for offensive aggression against animals from G3.

Aggression Testing

Animals from G1 and G2 were tested for aggressive behavior using the resident/intruder paradigm, a well-characterized and ethologically valid model of offensive aggression in golden hamsters (Floody and Pfaff, 1977; Lerwill and Makaings, 1971). Briefly, animals from G1 and G2 were presented in their home cage with an intruder (G3 animal) of equal age and weight and the resident hamster (G1 and G2 animals) was scored for aggressive behavior (i.e., number of attacks, bites, and latency to first bite vs an intruder) during a 10-min test period by two independent observers blind to the experimental treatment. In addition all animals were scored for flank marking behavior during the aggression tests. Flank marks were recorded each time the hamster arched its back and vigorously rubbed its flank gland against the side or wall of its cage while moving in a forward direction. All tests were performed during the first 4 h of the dark phase under dim red illumination. All encounters were videotaped for verification of the behavioral measurements. Following the tests for offensive aggression, animals from each treatment group (i.e., G1 and G2) were sacrificed by decapitation and the brains removed, frozen on dry ice-supercooled 2-methylbutane (Aldrich Chemical Company, Milwaukee, WI), and stored with desiccant at −80°C until use. Brain sections were then cut at 16 μm on a cryostat, thaw mounted onto Vectabond-treated (Vector Labs, Burlingame, CA) Superfrost plus slides (Fisher Scientific,

Pittsburgh, PA), and processed for *in situ* autoradiography.

In Situ Autoradiography and Image Analysis

Sections were processed for AVP V_{1A} receptor autoradiography using ^{125}I labeled linear AVP V_{1A} receptor ligand [HO-phenylacetly 1-D-Tyr(ME)2-Phe3-Gln4-Asn5-Arg6-Pro7-Arg8-NH$_2$ (New England Nuclear, Boston, MA, Catalog No. NEX310)] as previously described (Ferris *et al.,* 1993; Young *et al.,* 2000). Briefly, sections were preincubated in Tris buffer containing 100 μM NaCl and 100 mM guanosine 5-triphosphate (Type 11-S, Sigma Chemical Co., St. Louis, MO). Then, the sections were washed in ice-cold Tris containing 10 mM MgCl$_2$ and incubated in Tris buffer containing 10 mM MgCl$_2$, 0.01% bovine serum albumin (Fraction V, Sigma Chemical Co.), 0.05% bacitracin, 400 IU aprotinin, and 50 pM ^{125}I-AVP. Next, the sections were washed in ice-cold Tris buffer with 10 mM MgCl$_2$. After drying, the sections were exposed to Hyperfilm ^3H (Amersham Co., Arlington Heights, IL) along with autoradiographic ^{125}I microscale standards for 48–55 h.

To quantify the AVP V_{1A} receptor binding density in various brain regions, film autoradiograms were analyzed using a B95 Northern Light Illuminator (Imaging Research, Ontario, Canada), which provided uniform light distribution and intensity, connected to a Bioquant Nova 5.0 image analysis system (R&M Biometrics, Nashville, TN). The areas analyzed were selected based on data from previous studies implicating these regions in aggressive responding in numerous species and models of aggression, with the notable exception of the suprachiasmatic nucleus (SCN) and the S1 cerebral cortex (Ctx), i.e., nonaggression areas used as control regions. These areas (Fig. 1) included the medial division of the bed nucleus of the stria terminalis (BNST), the central amygdaloid nucleus (CeA), the corticomedial amygdaloid nucleus (CoMeA), the intermediate part of the lateral septal nucleus (LS), the lateral aspects of the medial preoptic area to anterior hypothalamus (MPOA-AH), the paraventricular hypothalamic nucleus (PVN), and the ventrolateral hypothalamus (VLH) which included the medial aspects of the medial tuberal nucleus and the ventrolateral part of the ventromedial hypothalamic nucleus. Based on values acquired from autoradiographic standards, the light intensity used to obtain AVP V1A receptor binding data was based on a linear illumination value. Background measurements were taken of the film and tissue (areas where AVP V_{1A} receptor binding was absent, e.g., the anterior com-
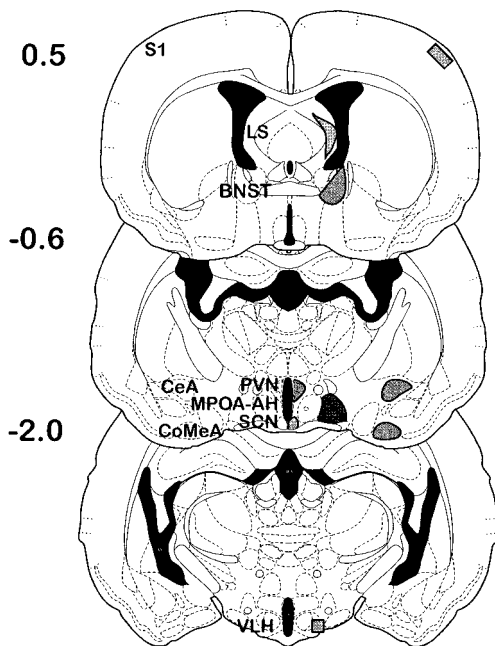
FIG. 1. Diagrams showing the location of the areas selected to quantify AVP V_{1A} binding densities (shaded areas). Diagrams were modified from the hamster atlas of Morin and Wood (2001). BNST, bed nucleus of the stria terminalis; CeA, central amygdaloid nucleus; CoMeA, corticomedial amygdaloid nucleus; LS, lateral septal nucleus; MPOA-AH, medial preoptic area to anterior hypothalamus; PVN, paraventricular hypothalamic nucleus; S1, S1 neocortex; SCN, suprachiasmatic nucleus; VLH, ventrolateral hypothalamus. 0.5 mm, −0.6 mm, and −2.0 mm from Bregma.

missure and corpus callosum). A standard region of interest was created to outline each brain region, and measurements for AVP V_{1A} receptor binding density were taken from each group. Specific binding was determined by subtracting the nonspecific labeling

(measurements from the anterior commissure or corpus callosum) from each brain area. Two to six independent measurements were taken from several consecutive sections of each animal ($n = 5$ or 6) per treatment group. The density of AVP V_{1A} receptor binding was then averaged for each brain region per animal and used for statistical analysis.

Statistics

Behavioral studies. The results from the aggression tests were compared between AAS-and vehicle-treated groups. Nonparametric data (number of bites, attacks, and flank marks) were compared by Mann–Whitney U tests (two-tailed). Parametric data (latency to first bite) were compared by Student's t test (two-tailed)

AVP V1A receptor binding. The density of AVP V1A receptor binding sites was compared between treatment groups by Student's t test (two-tailed) for each area analyzed.

RESULTS

Offensive Aggression

As observed previously (Harrison *et al.,* 2000; Melloni *et al.,* 1997; Melloni and Ferris, 1996), animals treated with AAS during their adolescent development showed significantly heightened measures of offensive aggression (Fig. 2). Adolescent AAS-treated hamsters showed a significant increase in the number of attacks ($Z = 3.04$, $P < 0.01$), bites ($Z = 3.05$, $P < 0.01$), and flank marks ($Z = 2.82$, $P < 0.01$) over sesame oil-treated control animals. Greater than 80%
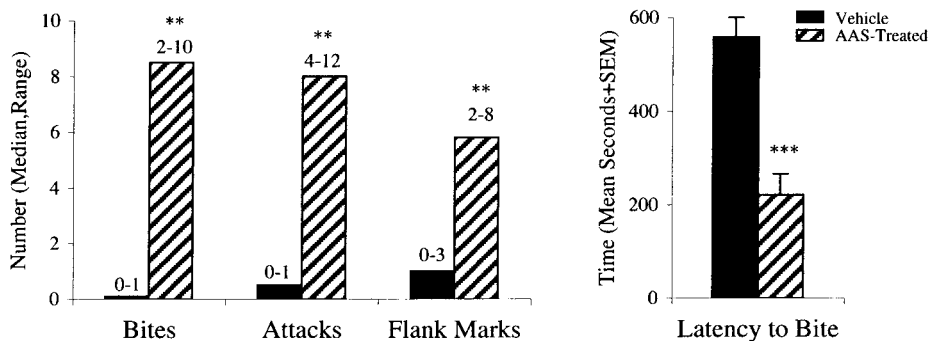

FIG. 2. Chronic adolescent anabolic steroid exposure facilitates offensive aggression. Median number of bites, attacks, and flank marks and mean latency to bite in control (vehicle, black bars) and anabolic steroid (AAS-treated, striped bars) residents. $n = 6$ animals/group. **$P < 0.01$, Mann–Whitney (bites, attacks, and flank marks). ***$P < 0.001$, Student's t test (latency to bite).
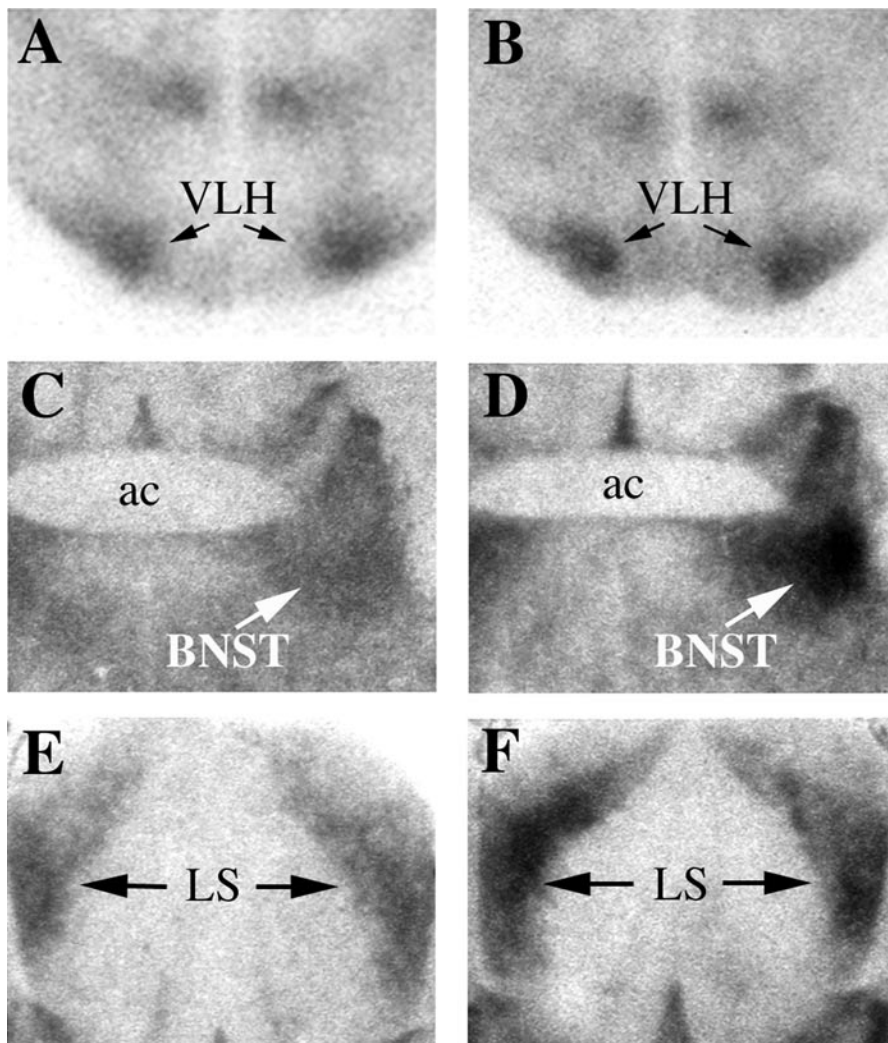
FIG. 3. Film autoradiographs of AVP VIA-selective radioligand binding in (A, C, E) vehicle-treated and (B, D, F) anabolic steroid-treated hamsters. Arrows indicate AVP VIA receptor binding in the (A, B) VLH, ventrolateral hypothalamus; (C, D) BNST, bed nucleus of the stria terminalis; and (E, F) LS, lateral septum of adolescent AAS-treated animals, ac, anterior commissure.

of the animals tested had more than five times the maximum number of attacks, bites, and/or flank marks of controls. Adolescent AAS-treated hamsters also displayed a significantly quicker aggressive response toward intruders (latency to bite, $t(10) = 5.58$, $P < 0.001$) than oil-treated controls. Here, adolescent AAS-treated hamsters were nearly three times faster to bite intruders than vehicle-treated controls.

AVP V1A Receptor Binding

In general, AVP V_{1A} receptor labeling in control animals was similar to that described previously in the adult hamster brain (Johnson *et al.,* 1995; Young *et al.,*

2000). AVP V_{1A} receptor binding was abundant in the VLH, LS, BNST, CeA, and SCN. Moderate labeling was found in the MPOA-AH, CoMeA, and PVN. Light to moderate labeling was observed in the S1 cerebral cortex.

In aggressive, AAS-treated hamsters, the density of AVP V_{1A} receptor binding was altered in several aggression areas of brain. For instance, in sesame oil-treated control animals, dense AVP V_{1A} receptor labeling was observed in the VLH indicative of the normal pattern of AVP binding onto neurons in this brain region (Fig. 3A). By comparison, aggressive, AAS-treated animals displayed markedly more binding for AVP V_{1A} receptors in the VLH brain region (Fig. 3B).
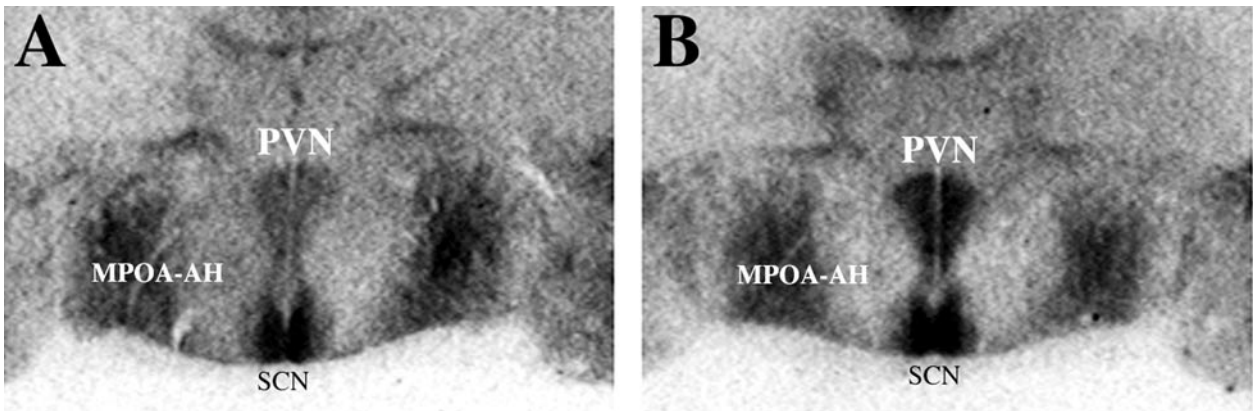
FIG. 4. Film autoradiographs of AVP VIA-selective radioligand binding in (A) vehicle-treated and (B) anabolic steroid-treated hamsters. PVN, paraventricular nucleus of the hypothalamus; MPOA-AH, lateral aspects of the medial preoptic area to the anterior hypothalamus; SCN, suprachiasmatic nucleus.

Analysis of binding density in this brain region showed that adolescent AAS-treated animals had significantly greater (~20%) AVP V_{1A} receptor labeling compared with vehicle-treated littermates [$t(76) = 2.92$, $P < 0.01$] (Fig. 5). These findings were not restricted to the VLH, however, as other areas of the brain implicated in aggression showed similar increases in AVP V_{1A} receptor binding following adolescent AAS exposure. Specifically, aggressive, adolescent AAS-treated animals showed greater than twofold increases in AVP V_{1A} receptor binding in both the BNST and the LS and a fourfold increase in binding in the PVN compared with vehicle controls (Figs. 3, 5). These differences in AVP V_{1A} receptor density were highly statistically significant between treatment groups [BNST, $t(72) = 5.20$; LS, $t(33) = 5.22$; PVN, $t(38) = 15.06$; $P < 0.001$ each comparison].

Not every aggression area showed changes in AVP V_{1A} receptor binding following adolescent AAS exposure. For instance, although a slight increase was noted in AVP V_{1A} receptor labeling in the CoMeA of AAS-treated animals compared with vehicle-treated controls, this comparison did not reach statistical significance ($P = 0.089$) (Fig. 5). Similarly, no significant differences were observed in AVP V_{1A} receptor binding in the MPOA-AH and CeA between adolescent AAS- and vehicle-treated controls ($P > 0.1$ each comparison) (Figs. 4, 5).

AVP V1A Receptor Binding in Nonaggression Areas

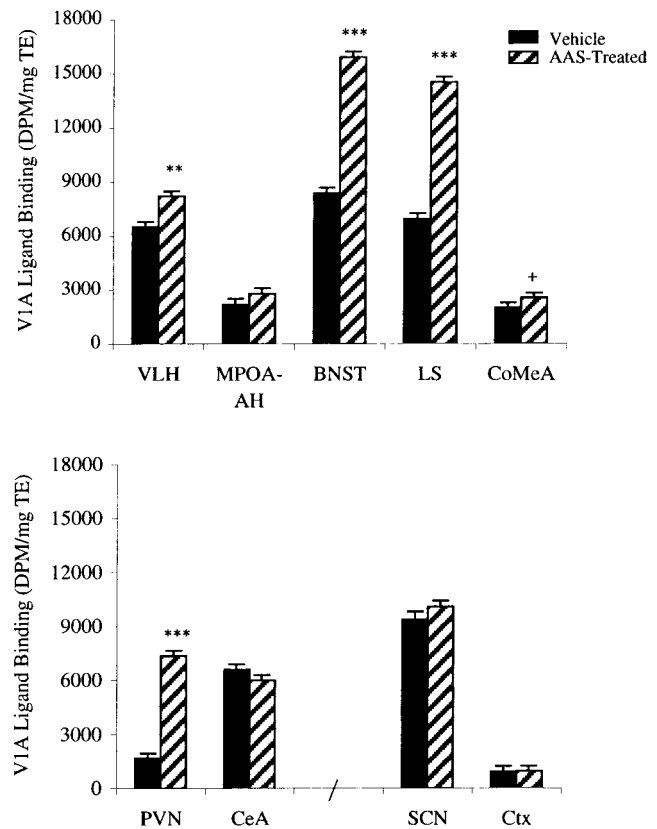No changes in AVP V_{1A} receptor binding were observed in several nonaggression control areas between

FIG. 5. Comparison of the density of AVP V_{1A} receptor labeling in brain areas of vehicle-treated (black bars) ($n = 5$ animals/group) and anabolic steroid-treated (striped bars) ($n = 6$ animals/group) hamsters. ***$P < 0.001$, **$P < 0.01$, †$P > 0.05$, and ***$P < 0.1$; Student's t test, two-tailed.

adolescent AAS- and vehicle-treated animals. More specifically, no significant differences were observed in AVP V1A receptor binding in the SCN nor the S1 cortex between AAS- and vehicle-treated animals ($P >$ 0.1 each comparison) (Figs. 4, 5).

DISCUSSION

In previous studies, we have shown that high-dose AAS treatment throughout adolescent development significantly increases offensive aggression in intact male Syrian hamsters (Harrison *et al.,* 2000; Melloni *et al.,* 1997; Melloni and Ferris, 1996). One mechanism by which chronic adolescent AAS exposure may facilitate offensive aggression in hamsters is by altering the activity of neurotransmitters implicated in this behavioral response. In hamsters, AVP has been shown to modulate offensive aggression by acting at the level of the MPOA-AH and VLH (Delville *et al.,* 1996a,b; Ferris *et al.,* 1989; Ferris and Potegal, 1988; Potegal and Ferris, 1989), where AVP acts to facilitate aggression through an interaction with the AVP V_{1A} subtype receptor (Delville *et al.,* 1996b; Ferris and Potegal, 1988). AVP V_{1A} receptor activity in these brain regions is testosterone-sensitive (Delville *et al.,* 1996b; Johnson *et al.,* 1995), with AVP V_{1A} receptor binding decreasing after castration, while being maintained by testosterone treatment. The reductions in AVP V_{1A} receptor binding observed following castration have been postulated to account for decreases in offensive aggression in hamsters (Albers, Liou, and Ferris, 1988; Ferris *et al.,* 1989). Conversely we hypothesized that exposure to AAS during adolescence might stimulate offensive aggression by increasing the binding of AVP to AVP V_{1A} receptors in these brain sites, functionally activating the neural circuits stimulating offensive aggression. To address this question, we quantified AVP V_{1A} receptor binding in the MPOA-AH and VLH in aggressive, adolescent AAS-treated hamsters versus vehicle-treated controls.

In the MPOA-AH, no ostensible differences were observed in the density of AVP V_{1A} receptor binding between aggressive, adolescent AAS-treated hamsters and nonaggressive vehicle-treated controls, suggesting that adolescent AAS-facilitated offensive aggression may not be modulated by alterations in the binding activity of AVP V_{1A} receptors in this brain region. However, recently we have shown that AVP afferent innervation and peptide levels are increased in the MPOA-AH of aggressive, adolescent AAS-treated hamsters (Harrison *et al.,* 2000), increasing the likelihood of enhanced AVP release and the subsequent activation of AVP V_{1A} receptors in these animals. Thus, it is probable that AVP signaling through the AVP V_{1A} receptor is increased in the MPOA-AH of adolescent AAS-treated hamsters in the absence of receptor upregulation, explaining the aggressive phenotype observed in our behavioral studies. Conversely, aggressive, AAS-treated animals showed nearly 20% increases in AVP V_{1A} receptor labeling in the VLH compared with vehicle-treated littermates, indicating increased AVP V_{1A} receptor binding activity in this brain area in response to adolescent AAS. Thus, it appears that high-dose testosterone treatment during adolescent development can overstimulate normative steroid signaling pathways in intact hamsters, upregulating AVP V_{1A} receptor binding activity in the VLH. From a functional standpoint, this increase in AVPergic tone may activate the VLH neural circuit implicated in the aggressive response, thereby stimulating offensive aggression. One potential mechanism through which adolescent exposure to AAS may increase VLH-AVP V_{1A} receptor binding is by increasing AVP V_{1A} receptor gene expression, perhaps by acting through the androgen receptor (AR). Indeed, testosterone has been shown to influence the expression of AVP V_{1A} mRNA (Young *et al.,* 2000). Further, the androgens used in these studies (i.e., testosterone, dihydrotestosterone, and nandrolone) bind avidly to the AR *in vitro* (Roselli, 1998), and ARs are abundant throughout the hamster hypothalamus, including the VLH (Clancy, Whitman, Michael, and Albers, 1994). In fact, the number of neurons expressing ARs and the intensity of AR immunostaining within neurons are both upregulated in the VLH in response to AAS (Menard and Harlan, 1993), providing a direct molecular mechanism for transcriptional activation of AVP V_{1A} gene expression in this brain area following adolescent AAS exposure. Further study examining the ability of AAS to regulate AVP V_{1A} receptor mRNA should be useful in determining the role of VLH-AVP V_{1A} receptor gene expression in adolescent AAS-facilitated offensive aggression.

Activity of neurons in the BNST, LS, and MeA has been implicated in the aggressive response in various species, including rats (Koolhaas *et al.,* 1990, 1991; Shibata, Yamamoto, and Ueki, 1982; Vochteloo and Koolhaas, 1987), mice (Gammie and Nelson, 2001), prairie voles (Wang, Hulihan, and Insel, 1997), cats (Han, Shaikh, and Siegel, 1996a,b; Shaikh and Siegel, 1994), and hamsters (Delville *et al.,* 2000; Kollack-Walker and Newman, 1995; Potegal *et al.,* 1996). AVP activity in two of these regions, namely, BNST and LS,

has also been shown to stimulate flank marking behavior in hamsters (Bamshad and Albers, 1996; Ferris *et al.,* 1994; Irvin *et al.,* 1990). Perhaps adolescent AAS exposure stimulates offensive aggression in hamsters by increasing AVP V_{1A} receptor binding on neurons located in these brain areas. AVP V_{1A} receptor activity in the BNST, but not the LS, has been shown to be testosterone-sensitive, with receptor binding decreasing as much as 25% after castration (Young *et al.,* 2000). And although AVP V_{1A} receptor binding in the LS is not testosterone-dependent (Young *et al.,* 2000), testosterone can influence the amount of flank marking stimulated by the direct application of AVP to the LS (Albers and Cooper, 1995), suggesting regulation of LS-AVP V_{1A}-mediated behavior by testosterone. In the present studies aggressive, adolescent AAS-treated animals showed greater than twofold increases in AVP V_{1A} receptor binding in both the BNST and LS compared with vehicle-treated controls. The enhanced AVP V_{1A} activity in the BNST and LS resulting from this increase in binding may cause the functional activation of neurons in this region, facilitating specific measures of offensive aggression. In particular, enhanced LS-AVP activity resulting from increases in AVP V_{1A} receptor binding following adolescent AAS exposure may explain the observed increases in flank marking in AAS-treated animals, while increased AVP V_{1A} receptor binding in the BNST may contribute to the activation of flank marking and attack behavior. Further studies examining the role of LS- and BNST-AVP V_{1A} activity in the various behavioral components of adolescent AAS-facilitated offensive aggression are currently underway in the laboratory.

Interestingly, compared with controls, aggressive, adolescent AAS-treated hamsters displayed greater than fourfold increases in AVP V_{1A} receptor binding in the PVN, i.e., an area of the brain not previously implicated in offensive aggression in hamsters. The PVN has been shown to modulate maternal aggression in rats (Consiglio and Lucion, 1996; Giovenardi, Padoin, Cadore, and Lucion, 1997, 1998), mice (Gammie and Nelson, 2001), and prairie voles (Gammie and Nelson, 2000), where PVN activity has been linked to both the inhibition (Giovenardi *et al.,* 1997, 1998) and activation (Consiglio and Lucion, 1996) of this behavioral response. In addition, the PVN participates in the modulation of quiet attack in cats (Schoel, Opsahl, and Flynn, 1981; Smith and Flynn, 1980). It is possible that adolescent AAS facilitates aggressive responding in hamsters by modulating AVP V_{1A} receptors in the PVN. Currently it is unknown which specific cell types in the PVN express AVP V1A receptors, al-

though these receptors have been colocalized on AVP neurons located in the supraoptic hypothalamic nucleus in rats (Hurbin, Boisson-Agasse, Orcel, *et al.,* 1998). It is possible that AVP V_{1A} receptors are upregulated in AVP neurons in the PVN also, in particular those that coexpress corticotropin-releasing factor (Kiss, Mezey, and Skirboll, 1984; Wolfson, Manning, Davis, Arentzen, and Baldino, 1985). This finding would indicate an important role for stress in adolescent AAS-facilitated offensive aggression. Additional study is needed to firmly establish the relationship between PVN AVP, AVP V_{1A} receptor activity, and stress in adolescent AAS-facilitated offensive aggression.

Lastly, to determine whether adolescent AAS exposure altered AVP V_{1A} receptor binding activity in non-aggression regions of the brain, we examined AVP V_{1A} receptor labeling in the S1 neocortex and the SCN, i.e., areas of the hamster brain that display testosterone-insensitive AVP V_{1A} receptor binding (Johnson *et al.,* 1995). In each case no significant difference in AVP V_{1A} receptor binding was observed between adolescent AAS- and vehicle-treated animals.

CONCLUSION

In summary, the studies presented in this paper provide the first data examining effects of chronic AAS exposure during adolescent development on the AVP V_{1A} receptor system regulating offensive aggression. These findings are novel in that they show that exposure to high-dose AAS during adolescent development can dramatically increase the binding of AVP V_{1A} receptors in intact animals. No studies to date have shown similar regulatory responses of the AVP V_{1A} receptor system to testosterone. These data are significant in that they show that increases in offensive aggression resulting from adolescent AAS treatment correlate directly with increases in AVP V_{1A} receptor binding activity in several areas of the hamster brain implicated in aggressive responding, but not in others. From a neuroanatomical standpoint, these data implicate enhanced AVP neural signaling via the AVP V_{1A} receptor in these aggression areas as potential neural substrates for adolescent AAS-facilitated offensive aggression.

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