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Anabolic-Androgenic Steroids and Brain Reward

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CLARK, A. S., R. C. LINDENFELD AND C. H. GIBBONS. *Anabolic-androgenic steroids and brain reward.* PHARMACOL BIOCHEM BEHAV 53(3) 741–745, 1996. – Anabolic-androgenic steroid (AAS) effects on brain reward were investigated in male rats with electrodes implanted in the lateral hypothalamus using the rate–frequency curve shift paradigm of brain stimulation reward. In the first experiment, treatment for 2 weeks with the AAS methandrostenolone had no effect on either the reward or performance components of intracranial self-stimulation. In the second experiment, treatment for 15 weeks with an AAS "cocktail" consisting of testosterone cypionate, nandrolone decanoate,and boldenone undecylenate did not alter brain reward but did produce a slight but significant change in bar press rate. In addition to the AAS treatment, animals in the second study were administered a single injection of *d*-amphetamine before and after 15 weeks of AAS exposure. The rate–frequency curve shift observed in response to a systemic injection of amphetamine was significantly greater in animals after 15 weeks of treatment with the AAS cocktail. Although AAS do not appear to alter the rewarding properties of brain stimulation, AAS may influence the sensitivity of brain reward systems.

Intracranial self-stimulation Androgen Amphetamine

ANABOLIC-ANDROGENIC steroids (AAS) are synthetic compounds taken in massive quantities by athletes with the intention of enhancing muscular appearance and/or physical strength [for reviews see (14,24)]. Misuse of AAS is becoming an increasingly prevalent substance abuse problem. Anabolic steroid dependence has been reported to develop with long-term AAS abuse in some individuals (4,12,23). The mechanisms by which AAS produce symptoms of dependence have only recently begun to be investigated (2). One paradigm that has been used extensively for assessing the reinforcing properties of drugs of abuse is the method of intracranial self-stimulation. Utilizing this approach, investigators have determined that specific compounds (e.g., *d*-amphetamine, cocaine) can produce reliable changes in self-stimulation behavior that can be characterized as changes in brain reward (21,25). There is a precedent for considering direct actions of steroid hormones upon brain reward systems (5,18,19). Furthermore, the interactions between steroid hormones and brain dopamine systems have been explored (16); however, few studies have assessed the effects of treatment with high levels of AAS on brain reward. In the present study, the rate–frequency curve shift paradigm of self-stimulation was used to evaluate the influence of two different AAS treatment regimens on brain reward systems (15,21). In addition, in the second experiment responsiveness to *d*-amphetamine was evaluated before and after AAS treatment.

METHOD

Subjects

Subjects for these experiments were adult male Long-Evans rats (approximately 90 days of age) housed individually with free access to food and water in the temperature-controlled animal colony in the Department of Psychology at Dartmouth College. The colony is maintained on a 12 L : 12 D cycle with lights on at 0600 h. Using aseptic technique, the rats were stereotaxically implanted with monopolar electrodes into the lateral hypothalamus (coordinates: 2.9 mm posterior to bregma, 1.7 mm lateral from the midline, and 8.2 mm below the cortical surface) under sodium pentobarbital anesthesia (50 mg/kg).

General Procedures

For both experiments, after a 1–2-week recovery period, the rats were trained to lever press in a standard operant cham-

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ber for a 1.0-s burst of 0.1-ms square wave monophasic constant-current pulses of brain stimulation. Stimulation trials were conducted daily between 0900 and 1200 h. The house light was illuminated during stimulation trials. In addition, a reinforcement light mounted above the response lever was illuminated each time brain stimulation was delivered. For each rat, the optimal current was determined as that current which yielded the highest rate of responding and the fewest signs of aversion. The lights, stimulation, and data collection were controlled using a Stimtek Co. stimulator connected to an IBM-PC.

The rats were initially trained on a continuous reinforcement schedule and then switched to a variable interval 3-s schedule. A stimulation current was selected for each rat that produced high rates of responding for a 63-Hz burst of stimulation. After consistent performance was achieved at the 63-Hz frequency, the animals were then given extinction/reacquisition training in which the stimulation frequency alternated between 1 and 63 Hz. The animals were then introduced to an ascending rate-frequency curve with a warm-up frequency of 63 Hz, extinction of 1 Hz, and increasing frequency parameters in 0.2 log unit increments ranging from 1.2 to 2.4 log Hz. A 10-s rest period separated the frequency increments. Each frequency condition was presented for a 2.5-min period. Bar press rates from the first 30 s of this period were excluded from the analysis to allow the rat time to adjust its responding to the new frequency. The average bar press rate during the remaining 2 min was considered the response rate for a given stimulation frequency.

Hormone and Drug Treatments

The effects of AAS on intracranial self-stimulation were investigated in this study using two separate treatment regimens. Analyses of the short-term (2 weeks) influence of AAS were conducted in rats that had established stable rate-frequency curves of no deviation from baseline greater than or equal to 0.1 log unit over the previous 5 consecutive days of testing [see (21) for discussion]. In our laboratory daily variations in locus of rise rarely exceed 0.02 log units. Five rats were used in Experiment 1. The subjects were treated daily with the AAS, methandrostenolone (1 mg/day, SC, n = 3), or the sesame oil vehicle (n = 2) 1 h prior to testing for 14 consecutive days. The AAS methandrostenolone was chosen for study based on its widespread human abuse and the dose was selected to mimic the human abuse range for this specific compound (4). The 2-week treatment period and 1-h interval between the drug injection and testing were selected based on other recent reports (5,8,19).

In a second experiment, eight male rats were used as subjects. These rats were treated with the AAS (n = 4) or oil vehicle (n = 4) for a period of 15 weeks. The longer treatment period was selected based on two factors. First, many human steroid abusers consume AAS for extended periods of time (24). Second, our laboratory has observed behavioral changes that emerge after approximately 3 months of AAS treatment (7). Because we did not observe a change in self-stimulation after administration of an individual AAS in Experiment 1, in the second experiment we elected to utilize an AAS "cocktail" reflecting the full spectrum of commonly abused AAS compounds. An AAS "cocktail" consisting of testosterone cypionate (2 mg/kg), nandrolone decanoate (2 mg/kg), and boldenone undecylenate (1 mg/kg) (TNB), or the sesame oil vehicle was administered 1 h before the stimulation session [(6), see (24) for further discussion of the chemical nature of these compounds].

Subjects in Experiment 2 were evaluated for their response to amphetamine before and after 15 weeks of treatment with the AAS or oil vehicle. Before the AAS treatment period began, the rate-frequency curve shift in response to a single injection of *d*-amphetamine sulfate (0.5 mg/kg, IP, 15 min prior to the testing session) was quantitatively determined for each rat. Rate-frequency curve shifts in response to a single injection of the same dose of *d*-amphetamine were measured again in the same animals after 15 weeks of treatment with the AAS or oil vehicle. The hypothesis was that the sensitivity of the brain to amphetamine treatment would be altered by AAS (i.e., that 15 weeks of treatment with AAS would elicit a larger shift in the rate-frequency curve after an injection of *d*-amphetamine).

Data Analyses

The rate of bar press was plotted against the log of pulse frequency to yield a rate-frequency curve. Two statistics are derived from the fitted curve. The locus of rise (LOR) denotes the pulse frequency required to sustain half-maximal responding. Quantifiable lateral shifts of the rate-frequency curve to the left, expressed as a decrease in LOR, represent an increase in the reward value of the stimulation. For statistical analyses, a lateral curve shift of the LOR of at least 0.1 log unit has been considered the minimum requirement for a change in reward (21). The second statistic is the maximum rate of bar pressing, referred to as MAX. A change in MAX is represented by a vertical shift of the rate-frequency curve, and is reflective of a selective impairment in motor/performance capacity (21). Difference scores (changes during the treatment period vs. baseline) calculated for LOR (dLOR) and MAX (% change) were compared between the AAS and oil groups using the Mann-Whitney *U*-test, whereas changes in LOR within animals before and after amphetamine administration were compared using Wilcoxon signed-ranks test.

RESULTS AND DISCUSSION

Treatment with the AAS methandrostenolone at a dose of 1 mg/day for 14 days produced no significant changes in brain stimulation reward. The AAS-treated group showed an average decrease in LOR of 0.019 log units, whereas the control group showed an average increase in LOR of 0.0175 log units relative to baseline (Mann-Whitney *U*, NS). Maximum bar press rates (MAX) did not differ between the groups as a consequence of hormone treatment (data not shown).

Similarly, 15 weeks of treatment with the AAS cocktail produced no significant changes in LOR (Fig. 1A). Neither the AAS or oil control group showed a significant lateral shift of the rate-frequency curve. In contrast, 15 weeks of treatment with the AAS cocktail did produce an ~6% decrement in MAX that was significantly different from the oil-treated control group ($p < 0.05$) (Fig. 1B).

The second experiment also evaluated the influence of AAS treatment on the rewarding value of amphetamine. Before beginning the 15-week AAS treatment phase, subjects displayed a parallel shift of the rate-frequency curve to the left of approximately 0.1 log units (Fig. 2A). Animals treated for 15 weeks with the AAS cocktail showed a significantly larger curve shift after the treatment period than during the baseline period (Wilcoxon signed-ranks, p = 0.03, one-tailed; Fig. 2A). There was no change in the lateral curve shift in response to amphetamine in the group receiving oil injections for 15 weeks. In addition, we directly compared the difference scores in response to *d*-amphetamine in the AAS-treated and oil-

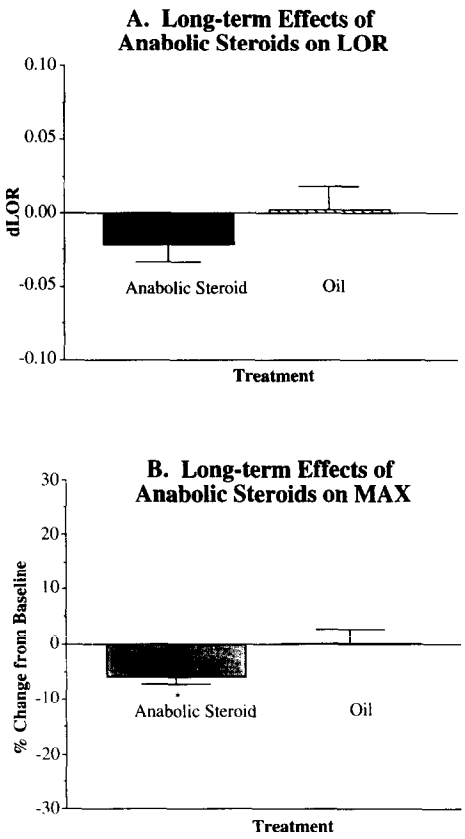

FIG. 1. (A) Animals treated with AAS daily for 15 weeks showed no significant change in LOR (dLOR) relative to the control group. (B) Animals treated with AAS for 15 weeks showed a significant decrease in the maximum rate of barpressing relative to the oil-treated controls (Mann-Whitney U, $^*p < 0.05$, two-tailed).

treated animals. Animals treated with AAS for 15 weeks displayed a marginally significant greater curve shift in response to amphetamine compared to the control group (Mann-Whitney U, one-tailed, $p = 0.057$). No significant changes in the maximum bar press rate after amphetamine injection were observed following the 15 weeks of AAS or oil treatment (Fig. 2B).

In the two experiments reported here, neither 2 nor 15 weeks of treatment with the individual AAS or the AAS cocktail, respectively, altered the rewarding properties of brain stimulation reward. No significant lateral shifts in the rate-frequency curve (LOR) were evident after either treatment regimen. These observations would seem to suggest that these specific AAS compounds, at the doses and time courses tested in the present experiment, did not alter brain reward directly. It is important to note that although the AAS utilized in the present study reflect those compounds that are widely abused, analyses of other compounds and dose ranges need to be explored before conclusions can be drawn regarding direct AAS effects on brain reward systems. In particular, the dose ranges selected for the present study were calculated based upon reports of human abuse level patterns (4). In this study we did not systematically explore the dose-response features of AAS effects on brain stimulation reward. Therefore, it remains a possibility that higher doses of AAS could have measurable effects on locus of rise values.

A limited number of studies have addressed the potential rewarding aspects of steroid hormones. Early experiments reported that rats showed changes in self-stimulation (as measured by bar press rate) after long-term androgen treatment, suggesting a direct effect on intracranial self-stimulation (5,18). More recently, experiments using the conditioned place preference paradigm as a measure of brain reward have demonstrated that androgens can induce a conditioned place preference in castrated (8) and intact (1) male rats. The results of the present set of experiments would appear to challenge the notion that AAS are directly reinforcing. Other experimental approaches, such as analyses of AAS effects on the self-administration of other drugs of abuse [e.g., (10,22)] must be pursued to gain a better understanding of how AAS influence brain reward systems.

Although no changes in bar press rate were observed after 2 weeks of AAS treatment, a small but significant depression in the maximum bar press rate was observed in Experiment 2 after 15 weeks of exposure to the AAS cocktail. Animals treated with AAS for 15 weeks showed a slight decrease in MAX compared to the control group. The decrease in bar press rate appeared to be due to a slight increase in the aversiveness of the stimulation at the higher stimulation frequencies. The appearance of drug-induced stereotypic behaviors

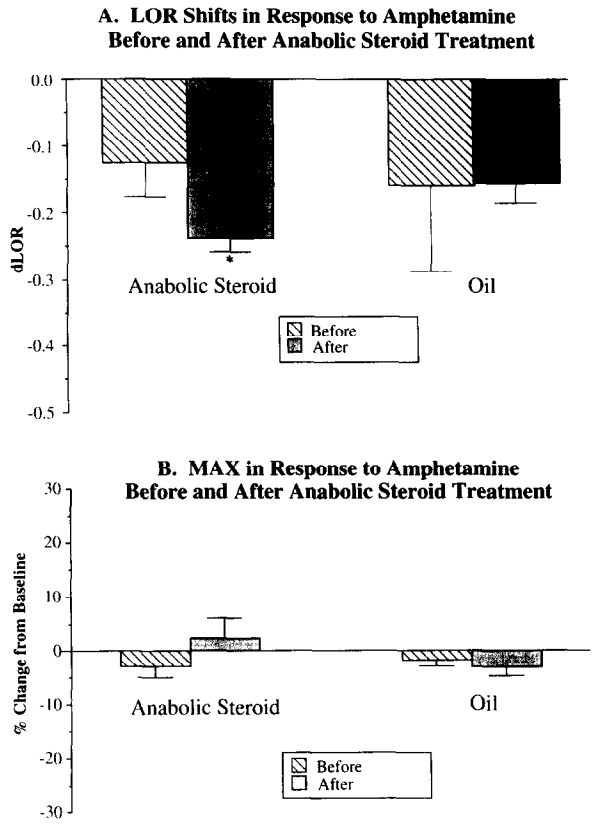

FIG. 2. (A) Animals showed a significantly larger shift in LOR in response to a single injection of d-amphetamine after treatment with AAS for 15 weeks, relative to the baseline period (Wilcoxon signed-ranks, $^*p < 0.05$, one-tailed). The control group showed no significant change in the LOR shift to amphetamine after 15 weeks of sesame oil treatment. (B) No significant alterations in MAX were observed at any time point in either treatment group in response to an acute injection of d-amphetamine.

at high stimulation frequencies has been reported previously, although in one study the decrease in bar press rate was reported to be accompanied by an increase in brain reward (17).

Experiment 2 investigated an additional parameter of responsiveness to AAS. It is known that low doses of amphetamine can produce a small change in the rewarding value of brain stimulation (11,15). This graded response has provided a tool for researchers to combine amphetamine with other substances to amplify treatment effects. That is, drugs that cause slight changes in reward may not produce marked rate-frequency curve shifts when tested alone, but when combined with amphetamine a more discernible shift may be observed (11,20). We adopted this approach to assess AAS effects on brain reward and determined the curve shifts in response to a single injection of amphetamine before and after 15 weeks of treatment with the AAS cocktail. We observed a significantly greater curve shift (a greater decrease in LOR) in response to a low dose of *d*-amphetamine (0.5 mg/kg) in animals after 15 weeks of exposure to the AAS cocktail. The effects of this low dose of *d*-amphetamine on the rate-frequency curve were exclusively on the LOR measures; no significant changes in MAX were detected. The observation that AAS treatment increased the magnitude of curve shift in response to *d*-amphetamine suggests that AAS act directly on the brain to increase the rewarding aspects of amphetamine. Long-term treatment with other psychoactive substances (*d*-amphetamine, for example) has been shown to sensitize brain reward systems (3). It may be that long-term AAS treatment produces a similar reward sensitization. Alternative interpretations of the increased curve shift after AAS treatment must also be considered. For example, AAS abuse can have adverse effects on liver function, which may in turn alter steroid metabolism (9,13). Thus, one consequence of the AAS treatments administered here may have been to retard the metabolism of amphetamine, resulting in sustained elevation of blood and brain levels of amphetamine in the AAS-treated animals. To rule out the possibility that the increased curve shift in response to amphetamine was due to AAS effects on peripheral metabolism, experiments need to be done in which the amphetamine is applied directly into the brain.

Perhaps AAS treatment did make the brain more sensitive to the administration of *d*-amphetamine. In this case, what mechanisms might underlie this heightened sensitivity? Much has been written about neural circuits that underlie brain stimulation reward. One pathway that is thought to be important for brain stimulation reward is the dopaminergic system (21,25). Presumably, the amphetamine treatments given in the present study altered brain reward, in part, via actions on central dopaminergic neurons. Steroid hormone effects on dopamine neurotransmission have been studied extensively; however, the role of androgens in regulating dopamine function has only recently been addressed (16). Further studies examining the impact of varied AAS treatments on the brain systems mediating reward may lead to a better understanding of the characteristics of these compounds that precipitate abuse.

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