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BRIEF COMMUNICATION

Behavioral Effects of Long-Term Administration of an Anabolic Steroid in Intact and Castrated Male Wistar Rats

DARIN M. MINKIN, MERLE E. MEYER AND FRANS VAN HAAREN[1]

Department of Psychology, University of Florida, Gainesville, FL 32611

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MINKIN, D. M., M. E. MEYER, AND F. VAN HAAREN. *Behavioral effects of long-term administration of an anabolic steroid in intact and castrated male Wistar rats.* PHARMACOL BIOCHEM BEHAV **44**(4) 959-963, 1993.—Once a week, intact and castrated male Wistar rats were intramuscularly injected with a 0.2 ml suspension of either 0, 10, or 50 mg nandrolone decanoate in cottonseed oil, for 8 consecutive weeks. After the sixth injection, locomotor activity was measured in an open-field and the acquisition of lever press behavior was assessed in an autoshaping procedure. Subsequently, all subjects were exposed to four sessions of continuous reinforcement prior to one session in which the effects of steroid administration on extinction were assessed. Locomotor activity decreased for all groups of rats with continued exposure to the open-field, differences between groups were not observed. Rats treated with the highest dose of nandrolone decanoate spent more time in the margin of the open-field. There were no significant differences between groups on any of the learning measures. Long-term, high-dose steroid administration in conjunction with mild food deprivation inhibited growth in intact and castrated rats, while low dose administration affected body weight in intact rats only. Steroid administration resulted in heavier and enlarged kidneys and lighter testes as well. These results suggest that the administration of anabolic steroids not only produces observable physiological changes, but that it may also affect spontaneous behavior. The failure to find differences in learning indices may have been a function of the particular paradigms used in the present experiment.

Anabolic steroids Nandrolone decanoate Drug abuse Body weight Locomotor activity
Autoshaping Extinction Lever press Rats

SINCE the chemical characterization of testosterone in 1935 more than 1000 derivatives have been synthesized (1). Chemical modification of the testosterone molecule has been achieved to increase its anabolic effects, which include increased total body nitrogen retention, increased number of red blood cells, and improved calcium deposition in bones and muscle enlargement. Anabolic-androgenic steroids are used in human medicine for the treatment of growth disorders, cancer, and certain blood disorders, and in veterinary medicine and animal husbandry (for review see 4). Over the last few decades anabolic steroids have also become increasingly popular with body builders, powerlifters, football players, and other athletes from a variety of sports as a means of gaining muscle size, strength, speed, and improved overall athletic performance. In fact, the abuse of anabolic steroids now appears to be widespread and has reached an alarming level (6,15).

It is generally accepted that the use of anabolic steroids is associated with a variety of harmful side effects. Reports in the popular press have associated long-term steroid abuse with cancers in well-known athletes. It has also been reported that, anabolic steroid abuse in humans is accompanied by increased cardiovascular disease risk profiles, cerebral dangers, psychosis, and schizophrenic disorders (4). In animal studies attention has mostly been focused on some of the physiological effects of steroid administration. For example, it has been shown that rats treated with a high dose of nandrolone decanoate (100 mg/kg) had heavier kidneys and lighter testes and livers than rats that had not been treated. Steroid treatment also inhibited body growth (16).

As other dangers of long-term steroid abuse have only recently been recognized, very little attention has been paid to the effects of anabolic steroids on an organism's behavior, although reports have circulated to suggest that aggressive

[1] To whom requests for reprints should be addressed.

behavior may be increased in both humans and animals who have been exposed to excessive quantities of anabolic steroids (e.g., 3, 8, 11).

The present experiment was designed to investigate whether or not chronic administration of different doses of an anabolic steroid (nandrolone decanoate) would affect the behavior of intact and castrated male Wistar rats in a variety of experimental procedures. All subjects were, first of all, exposed to an automated open-field to measure the effects of steroid administration on exploratory behavior, which previously has been shown to be influenced by steroid hormones as male rats are frequently less active than female rats (13, for review). The time spent in the margin of the open-field was also evaluated because some investigators have argued that the amount of time that animals spent away from the center of the open-field provides a good indication of anxiety, which may be affected by chronic steroid abuse (1,7). Subsequently, all subjects were trained to retrieve food pellets from a magazine in an operant chamber, prior to exposure to an autoshaping procedure in which they were to learn to press a lever through association of the illumination of the stimulus lights above the lever (conditioned stimulus, [CS]) with pellet presentation (unconditioned stimulus, [US]). Previous research has suggested that the rate of response acquisition in these procedures may be functionally related to the activity of steroid hormones as males are more likely than females to press a lever whose presentation has consistently been followed by the presentation of response-independent food (14).

Subsequently, all subjects were trained on a schedule of continuous reinforcement in which each lever press was immediately followed by food for four sessions, in preparation for one session in which lever pressing was put on extinction. Previous research has suggested that the speed of extinction of operant (and other behavior) may be negatively affected by the presence of male gonadal steroids (9). Intact and castrated male Wistar rats participated in the present experiment to investigate whether or not the presence or absence of endogeneous gonadal hormones in addition to exogeneous anabolic steroid administration would differentially affect behavior and body physiology.

METHOD

Subjects

Thirty-six male Wistar rats were obtained from a commercial breeder (Charles River, Wilmington, MA) when they were approximately 90 days old. They were individually housed in stainless-steel suspended cages upon arrival in the laboratory under a reversed light–dark cycle (lights on 6:00 p.m. to 6:00 a.m.). After 1 week of adaptation to the laboratory conditions, subjects were randomly assigned to the different experimental groups. Eighteen subjects were castrated under metofane anesthesia, while the remaining 18 subjects received sham surgery. All subjects were allowed free access to food and water until 2 weeks prior to the start of behavioral testing when they were all placed on a restricted diet until the end of the study. Access to food was limited to 1 h immediately following the experimental session (5).

Steroid Administration. Starting 3 weeks after surgery and continuing once a week for 8 consecutive weeks, different subjects were given intramuscular injections of 0, 10, or 50 mg/0.2 ml nandrolone decanoate (Sigma, St. Louis, MO) dissolved in cottonseed oil. Injections were continued during behavioral testing to insure consistent steroid exposure for the duration of the experiment. The following six groups of subjects (n = 6) participated in the experiment: 1) intact, oil only; 2) intact, low dose; 3) intact, high dose; 4) castrated, oil only; 5) castrated, low dose; and 6) castrated, high dose.

Apparatus

Activity Monitors. Four Digiscan activity monitors (Omnitech Electronics, Columbus, OH) were used in this experiment. These monitors, which have been described in more detail elsewhere (7), consist of a Plexiglas activity cage (40 × 40 × 30.5 cm) surrounded by horizontal and vertical infrared beams. For the purposes of the present study, special attention was paid to the following two dependent measures: 1) total distance, defined as the total distance travelled by an animal in centimeters; and 2) margin time, defined as the time spent by an animal in close proximity (within 1 cm) to the walls of the monitor.

Operant Chambers. Six, identical, Coulbourn Instruments, (Lehigh Valley, PA) modular rodent-operant-conditioning chambers, which have been described in more detail elsewhere were also used in the present experiment (12). All experimental chambers were housed in individual sound-attenuating, ventilated cabinets. The chambers were connected to a PDP 11-23 microcomputer (Digital Equipment Corporation, Maynard, MA) located in the experimental room itself. Experimental contingencies and data acquisition procedures were programmed using SKED-11 (10), obtained from State Systems, Inc. (Kalamazoo, MI).

Procedure

Experimentation was started the day following the sixth steroid injection. Sessions were run 7 days a week and took place during the subject's dark hours.

Locomotor Activity. Subjects were exposed to the activity monitor for 1 h during which total ambulation (in cm) and margin time (in s) were recorded every 15 min.

Magazine Training. Subjects were trained to retrieve 40 food pellets from the magazine in the operant chamber which were automatically delivered on a variable-time (VT) 40-s schedule. Magazine training was considered to be complete when a subject had consumed all pellets at the end of two consecutive sessions.

Autoshaping. Upon completion of the magazine training all subjects were exposed to an autoshaping procedure for eight consecutive sessions. The houselight was illuminated at the start of each session. Then, the stimulus lights above the left lever (CS) were illuminated after the expiration of an intertrial interval (ITI) of 50 s. The stimulus lights were extinguished after 10 s and response-independent food (US) was immediately presented. All food presentations were accompanied by the illumination of the light in the food tray for 2 s. Sessions were terminated after 40 CS-US pairings had been presented.

Continuous Reinforcement (CRF). Starting the day following the completion of the eigth autoshaping session, all subjects participated in four daily sessions during which the houselight as well as the stimulus lights above the left lever were illuminated at the start of the session and each lever press was immediately followed by a food pellet. Each session was terminated after 40 pellets had been collected or after 30 min whichever came first.

Extinction. The day after the fourth CRF session, all subjects were exposed to a single session in which lever presses were no longer followed by food presentation. This extinction

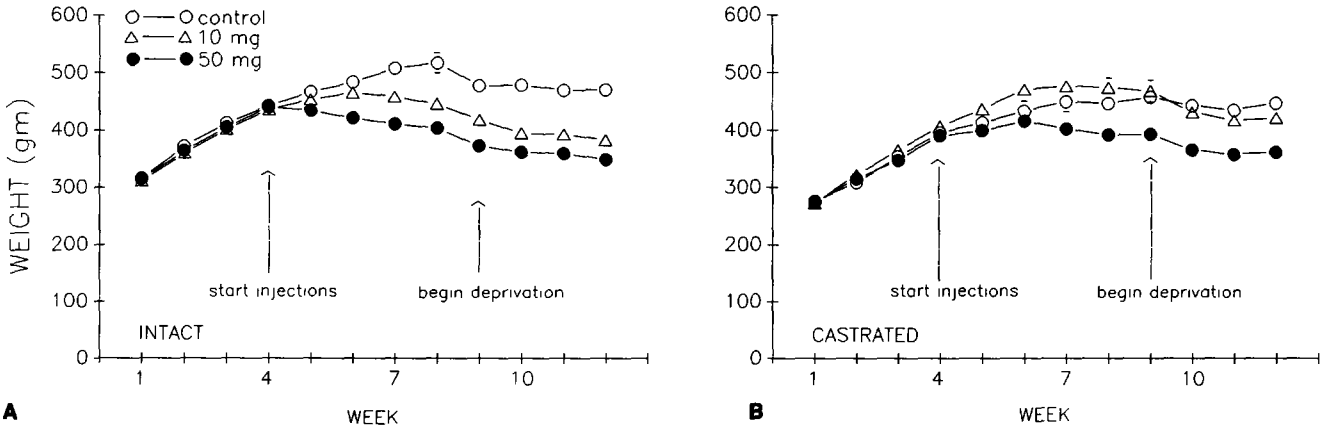

FIG. 1. Body weight development (in grams, ± one SE) for the different groups of subjects during the course of the experiment [(A) = intact subjects; (B) = castrated subjects].

session was terminated once the subject had not pressed the lever for 5 min or after 30 min, whichever occurred first.

Physiology. Prior to and during behavioral testing the subjects' body weights were closely monitored. Upon completion of behavioral testing, all subjects were given lethal injections of sodium pentobarbitol and the weights of the heart, kidneys and testes were recorded.

RESULTS.

Figure 1 shows body weight development for subjects in the different treatment conditions during the course of the experiment (lefthand panel, intact subjects, righthand panel, castrated subjects).

Weight gain did not differ between groups of subjects prior to the start of steroid injections. During the administration of nandrolone decanoate intact treated subjects did not gain as much weight as control subjects. Similar observations were made in the groups of castrated subjects except for the fact that steroid administration only inhibited body growth in the group of castrated subjects treated with the higher dose of nandrolone decanoate (Drug Dose,[$F(2, 35) = 25.61$, $p < 0.01$]; Hormonal Status × Drug Dose, [$F(2, 35) = 4.21$, $p < 0.01$]. Analysis of variance only included the weeks during which nandrolone decanoate was actually administered.

Figure 2 shows the total distance travelled by the different groups of subjects (upper panel) and the time spent in the margin (lower panel) during individual 15 min segments of the 60-min observation period in the open field.

Locomotor activity decreased for all groups of subjects with prolonged exposure to the activity monitor. Significant differences between groups were not observed. Subjects spent different amounts of time in the vicinity of the outer edges of the activity monitor dependent upon treatment condition. Margin time increased during the session [Time, $F(3, 108) = 8.29$, $p < 0.01$] and intact, as well as castrated subjects that were being treated with the higher dose of nandrolone decanoate spent more time in the margins of the monitor than other subjects [Drug Dose, $F(2, 35) = 3.21$, $p < 0.05$].

The results obtained during the different learning sessions varied little between groups of subjects. During magazine training, most control subjects learned to retrieve all 40 pellets from the feeder within two or three sessions (Intact, 2.67; Castrated, 2.83 sessions). Intact treated rats required an average of 3.67 (low dose) and 3.67 (high dose) sessions, while castrated treated rats averaged 4.5 and 3.5 sessions for magazine training to be completed. Hormonal condition and drug treatment did not significantly affect behavior during autoshaping sessions as the number of lever press responses was low for all groups of subjects, including control subjects. During exposure to the CRF condition only a few subjects failed to obtain the maximum number of pellets during any of the four sessions (three of the castrated, oil treated subjects, one

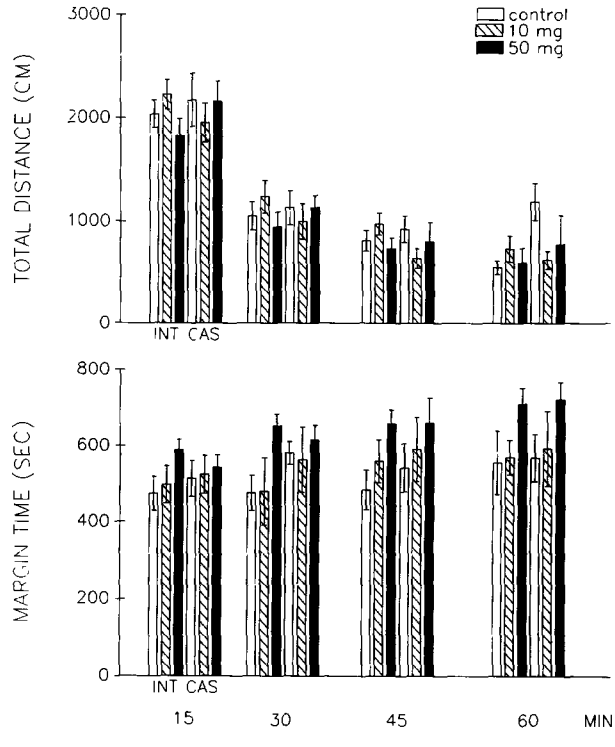

FIG. 2. The total distance travelled (in centimeters, ± one SE) by the different groups of subjects and the margin time (in seconds, ± one SE) during individual 15-min segments of the 60-min session. INT = intact and CAS = castrated subjects, respectively.

of the castrated subjects treated with the low dose of nandrolone decanoate and two of the intact subjects treated with the high dose of nandrolone decanoate). Differences during extinction were also not observed (data not shown).

Figure 3 shows the wet weights of the hearts, kidneys, and testes (intacts only) for the different groups of subjects. Differences in the weight of the hearts were not observed, but the hearts of treated animals, especially those treated with the higher dose of nandrolone decanoate appeared smaller and harder than the hearts of the control subjects. The kidneys of the treated animals were all much heavier than those of the control animals [Drug Dose, $F(2, 35) = 60.27$, $p < 0.01$]. Only the testes of those subjects treated with the low dose of nandrolone decanoate were significantly lighter than those of the control subjects [$t(10) = 3.75$, $p < 0.01$].

DISCUSSION

The present experiment was designed to assess the effects of chronic treatment with an anabolic steroid (nandrolone decanoate) on simple measures of adaptive behavior in intact and castrated male Wistar rats. The results of the experiment are interesting for a number of reasons. First of all, they provide some, albeit preliminary, indication that the chronic administration of anabolic steroids not only changes body physiology, but that an individual's behavior may also be affected. Of course, (anecdotal) evidence has long been available to indicate that the use of anabolic steroids increases agressive behavior in humans (3), and it is well-known that steroid administration facilitates the observation of aggressive interactions in laboratory animals (8). Thus far, very little research effort has been aimed at identifying possible other (harmful) side effects of long-term anabolic steroid abuse on human behavior, not in the least because of the fact that the debilitating physical effects of chronic steroid administration have prevented the use of appropriate research designs.

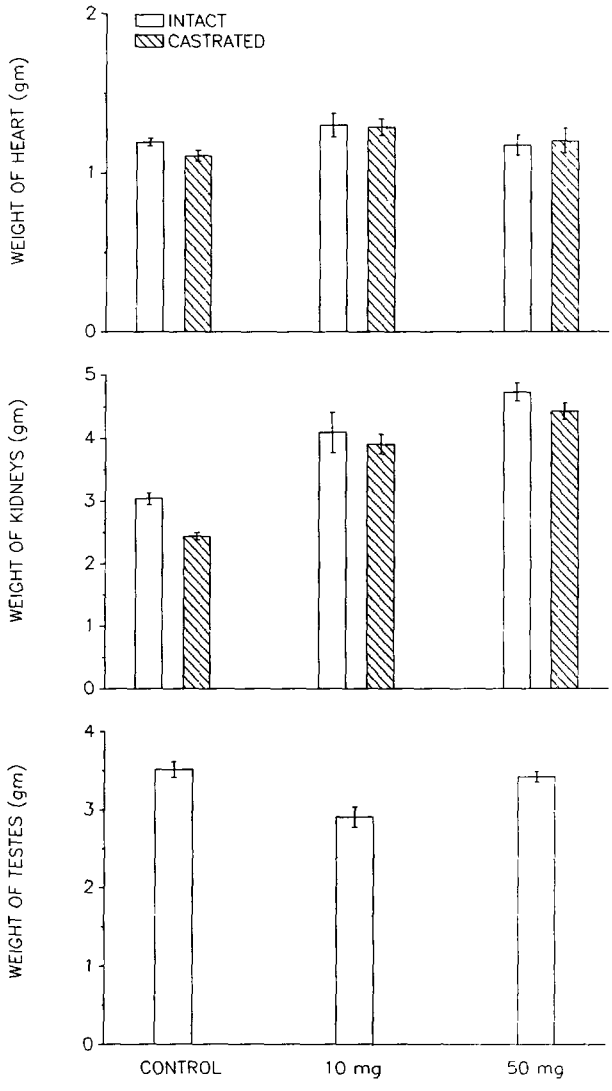

FIG. 3. Post-mortem wet weights of the heart, kidneys, and testes (in grams, ± one SE) in the different groups of subjects.

Now that it has been recognized that steroid self-administration may be a function of many of the same variables that have also been identified as involved in the initiation and maintenance of other drug abuse, a behavioral pharmacology of steroid abuse using animal models has become inevitable. The present observations suggest that chronic anabolic steroid administration may, in fact, have behavioral sequelae. Chronic administration of a relatively high dose of nandrolone decanoate induced subjects to spend more time hugging the walls of an activity monitor than subjects who had not been treated or treated with a lower dose. Thigmotaxis has been suggested to be indicative of increased anxiety (7). Few differences between treatment groups were observed during those experimental conditions designed to tap into different aspects of the subject's learning repertoire. The absence of differences during autoshaping sessions may have been due to the low level of response acquisition even in control subjects. The fact that nonretractable levers were used instead of retractable levers as in other studies (14) may bear on this issue. Behavioral differences were also not observed in four sessions of continuous reinforcement and one extinction session, maybe due to the fact that the transition from reinforced to nonreinforced behavior was too easily discriminated under the experimental parameters used in the present study.

The results of this experiment indicate that long-term administration of different doses of nandrolone decanaote in conjunction with mild food deprivation inhibited growth in intact and castrated male Wistar rats receiving a high dose of the anabolic steroid. Low dose administration affected body weight in intact rats only. In agreement with previous findings it was observed that long-term administration of nandrolone decanoate resulted in heavier and enlarged kidneys in treated rats. Testes were only lighter in those subjects treated with the low dose of nandrolone decanoate. It is, at present, unclear whether or not the absence of a difference in testes weight between oil-treated rats and rats treated with the higher dose of nadrolone decanoate needs to be attributed to measurement errors. Differences in heart weight were not observed, but informal observations suggest that the hearts of those subjects who were administered the high dose of nandrolone decanoate were much smaller, denser and harder.

In summary, the results of the present experiment offer some support for the suggestion that chronic administration of an anabolic steroid not only inadvertently affects important bodily functions, but that it may also interfere with behavior other than aggressive behavior, albeit that the present evidence is not overwhelming. The present data do suggest, however, that it may be pertinent to award the study of steroid abuse the same importance as the study of other abused substances

to help identify the behavioral variables and physiological mechanisms that may be involved in the initiation and maintenance of steroid self-administration, and the development of tolerance and sensitization, to name but a few (see also, 2).

ACKNOWLEGEMENTS

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