Effects of Prolonged Exposure to Anabolic Steroids on the Behavior of Male and Female Mice

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BRONSON, F. H. 1 Effects of prolonged exposure to anabolic steroids on the behavior of male and female mice. PHARMA-
COL BIOCHEM BEHAV 53(2) 329-334, 1996.—Adult male and female mice were exposed to a combination of four
anabolic-androgenic steroids at pharmacological doses for 6 months. Males were exposed to either 5 or 20 times androgenic
maintenance levels, as determined by bioassay; females were exposed to either one or five times the maintenance levels for
males. Even the low doses were sufficient to reduce gonadal weight in both sexes and eliminate the estrous cycle in females.
Steroid treatment blocked ejaculation in most males without influencing other facets of their sexual behavior. In some females
steroid treatment elicited male-like mounting and pelvic thrusting. Exposure to steroids increased aggressiveness in females.
Using a different testing protocol, steroid exposure had no effect on aggressiveness in males. Steroid treatment decreased
the use of a running wheel significantly in females and marginally in males. Overall, the results of this experiment suggest no
enhancement of normal androgen-mediated behavior in males, but potent effects on the behavior of females.

Anabolic-androgenic steroids Sexual behavior Ejaculation Aggression Running wheel activity Mice

DESPITE being banned by a variety of administrative entities, anabolic-androgenic hormones are widely used by male ath-
letes and body builders in an effort to improve their perfor-
manence or appearance, often starting in high school (28,32).
Some female athletes and body builders also use steroids, also
starting as early as high school, but the frequency with which
this occurs is much less well established [e.g., (16,25)].
Males often take as many as five different steroids simulta-
neously at doses that can total 10 to 40 times normal mainte-
nance levels, and they usually do this in cycles, starting weeks
or months before a competition, after which they initiate a
“wash-out” period of weeks or months (17,22). Little is known
about the doses or schedules used by females except that
women also take as many as five steroids simultaneously, also
at pharmacological doses, and a degree of masculinization of
secondary sexual characteristics can occur in female users (25).
Pharmacological doses of anabolic steroids can have a vari-
y of secondary effects, some of which are pathologic [re-
viewed, among others, by (12)]. Of particular interest here are
the potential effects of these steroids on sexual and aggressive
behavior. Most, but not all, of the available data suggest an
increase in aggressiveness due to steroid use in men [e.g., (3,
11,23,26,31); cf. (2,29)]. Likewise, some studies have re-
ported an increase in sexual activity among men taking ste-
roids (21) whereas others have found little if any effect [e.g.,
(4)]. Unfortunately, the available evidence in relation to both
kinds of behavior is only correlational and self-reported. Little
is known about the behavioral side effects of steroid abuse in
women.

The general objective of the present research was to explore
the effects of anabolic-androgenic steroids on the behavior of
mice using the number, kinds, and relative amounts of hor-
mones commonly used by human athletes and body builders.
The results of a previous study from this laboratory in which
female mice were exposed to anabolic steroids for a relatively
short period of time suggested that these hormones increase
aggressiveness and decrease spontaneous activity (8). As well
as testing the generality of these findings in females, the spe-
cific goals of the present study were twofold: to see if exposure
to pharmacological amounts of anabolic steroids would also
increase aggressiveness and decrease spontaneous activity in
males, and, second, to make a detailed exploration of the
effects of pharmacological amounts of anabolic steroids on
the sexual behavior of males. The results seen in males and
females in this study cannot be compared directly because the
doses used were different for the two sexes.
Animals and Experimental Design

Male and female CF-1 mice were purchased from Charles River at 7 weeks of age. Upon arrival they were housed one per polyethylene cage (29 x 18 x 12 cm), fed PMI Formulab 5008 chow, and maintained on a 14 L:10 D light cycle. At 8 weeks of age the animals were weighed and delegated to one of three weight-matched groups of 20 animals of each sex. One treatment group of each sex was given a low dose of a combination of four anabolic steroids; a second group was given a high dose of the same combination of four steroids; and a control group received no exogenous hormones. The absolute doses differed for the two sexes and in some cases the measurements made were different also. Thus, the research to be reported here must be visualized as entailing a 1 x 3 cell factorial that includes the two sexes in the same experimental design.

The hormones administered were testosterone, testosterone cypionate, methyltestosterone, and norethandrolone. The latter three are analogues of testosterone that span much of the variation that has been introduced to prolong potency or allow variation that has been introduced to prolong potency or allow treatment rather than their anabolic potency, which may not be identical at pharmacological levels (15). For females the low dose was a combination of the four hormones that would maintain normal-sized seminal vesicles in a castrated male CF-1 mouse (defined here as the maintenance dose) and the high dose was five times the low dose. For males the two doses were 5 and 20 times normal maintenance levels.

Steroid Administration

Each of the four hormones noted above was administered in its own Silastic capsule that was implanted under the skin on the back of a mouse. These capsules were packed solidly with crystalline hormone. Silastic capsules are characterized in terms of three dimensions: inner diameter, outer diameter, and length. The rate of release of a hormone from a Silastic capsule is proportional to the length of the capsule for any given set of diameters, and the rate of release remains constant over periods of time measured in months (6,10). Typically, dose-response curves using Silastic capsules are produced by varying capsule length while keeping the inner and outer diameter of the capsules constant.

The sizes of capsules to be used in these experiments were chosen with two goals in mind: to combine the four hormones noted earlier in such a way that each would contribute one-fourth of the total androgenicity at a given dose and, second, to keep capsule length as short as possible to minimize interference with an animal's behavior. To accomplish these goals it was necessary to vary all three dimensions of the capsules depending on the dose desired and the hormone to be administered.

The choice of the best capsule sizes to use in these experiments proceeded in two steps. First, the maintenance "dose" of each hormone was determined for capsules of different diameters by systematically varying capsule length. The results were assessed by bioassay. Maintenance dose was defined for each hormone as the capsule size required to maintain the seminal vesicles of a castrate male mouse at a weight typical of intact males. In separate studies for each of the four hormones, adult CF-1 males were castrated, immediately given hormone implants of various diameters and lengths, and then killed 2 weeks later at which time the stripped weight of the seminal vesicles was obtained. For comparison, each of these studies also included a group of castrated males given only blank capsules to establish baseline and a group of intact males to establish the normal weight of the seminal vesicles. As an example, Fig. 1 shows that the maintenance dose of testosterone was found to be either 2 mm of a 0.062" (inner diameter) x 0.125" (outer diameter) capsule or 10 mm of a 0.062" x 0.125" capsule. The other three hormones of interest were subjected to the same kind of analysis.

The second step was to determine if one could expose mice to all four hormones simultaneously with predictable results based on the capsule size of each. Castrated males were given the following four implants: 2.5 mm of a 0.062" x 0.125" capsule containing testosterone; 5 mm of a 0.062" x 0.125" capsule of testosterone cypionate; 2.5 mm of a 0.025" x 0.047" capsule of methyltestosterone; and 7.5 mm of a 0.062" x 0.125" capsule of norethandrolone. In each case these capsule lengths were one-fourth that shown to produce a maintenance dose when the hormone was administered alone in a capsule of the stated diameters. At autopsy 2 weeks later, the mean seminal vesicle weight of the animals given the four hormones as a combination was 88.2 ± 4.0 mg compared to 84.8 ± 5.7 for intact males and 23.0 ± 2.3 for castrates given blank implants (N = 8 in all cases). This combination of capsule sizes was defined as the maintenance dose for the combination of four hormones.

In the major experiments then, the maintenance dose defined above was the low dose given to females. The fivefold maintenance dose used as the high dose for females and the low dose for males combined the following: 12.5 mm of a 0.062" x 0.125" capsule containing testosterone; 5 mm of a 0.078" x 0.125" capsule of testosterone cypionate; 12.5 mm of a 0.025" x 0.047" capsule of methyltestosterone; and 7.5 mm of a 0.078" x 0.125" capsule of norethandrolone. This combination of capsules produced a mean seminal vesicle weight in the study described in the preceding paragraph of 112.8 ± 4.9 mg. The 0.025" x 0.047" capsule containing methyltestosterone had to be replaced after 4 months.

The 20-fold high dose in males combined: 10 mm of a 0.078" x 0.125" capsule of testosterone; 20 mm of a 0.078" x 0.125" capsule of testosterone cypionate; 10 mm of a 0.062" x 0.125" capsule of methyltestosterone; and 30 mm

FIG. 1. Bioassay of the amount of testosterone released from Silastic capsules of two diameters: 0.062" (inner diameter) x 0.125" (outer diameter), indicated by the solid line, and 0.078" x 0.125", indicated by the dashed line. The large circles indicate the capsule lengths that release a maintenance dose of testosterone for a normal intact male.
of a 0.078" x 0.125" capsule of norethandrolone. Control mice in all cases received implants of empty capsules.

Testing

At 8 months of age, after 6 months of exposure to steroids or blank capsules, the 120 mice were subjected to a series of tests over a 4-week period. The tests that were administered varied both among individuals and between the sexes.

Spontaneous use of a running wheel. Ten randomly chosen individuals of each sex of each treatment group were housed in 26 x 46 x 20 cm polyethylene cages, each containing a running wheel with a diameter of 16 cm and a tread width of 8 cm. The number of revolutions of the wheel was recorded and stored on a computer in 10-min bins using a Dataquest III Data Acquisition System (Mini-Mitter, Sun River, OR). After 5 days of habituation the amount and daily pattern of use of the running wheel was monitored for a 5-day period, after which the animals were returned to their smaller home cages. Interruption of an animal’s daily activity cycle to obtain vaginal smears was deemed inadvisable; thus, the stage of the estrous cycle of the control females was not assessed.

Male sexual behavior. Following the procedures outlined in Huber et al. (14), sexual behavior was assessed in the 10 males of each treatment group not housed in running wheel cages. Thirty- to 35-day-old CF-1 females were primed with pregnant mare serum and human chorionic gonadotropin (Sigma, St. Louis, MO). As before (14), the sexual behavior of the test males was quantified beginning 8 h after the females were given HCG. One hormonally primed female was placed in a male's home cage and left for 1 h or until ejaculation occurred. If a male did not achieve ejaculation with the first female, it was replaced with a second female, again for 1 h or until ejaculation occurred. Latency to mount, achieve intromission, and ejaculate were recorded, as was the number of mounts and intromissions. Three observers assessed the sexual behavior of the 30 males used in this study. This was done blind and the 10 males observed by each person included similar numbers of individuals from each of the three treatment groups.

Estrous cycle. Prior to the last test—the aggression test—vaginal smears were obtained from all 20 females in each treatment group for a minimum of 10 days. This was done to assess the effects of the hormone treatments on the estrous cycle and, secondly, to ensure that aggression testing was done only when control females were in metestrua or early diestrua (as will be detailed below, no steroid-treated female showed vaginal cycling).

Aggression. The protocol for assessing aggressiveness differed for the two sexes because male and female mice differ markedly in the frequency and viciousness with which they fight. A fight erupts relatively quickly when two previously isolated males meet for the first time, and it is often impossible to determine which male initiated the fight. Furthermore, most of the time one cannot determine the winner of an encounter between two males without letting them fight for several minutes, and this often results in wounding. Thus, the protocol for assessing aggressiveness in males in these experiments involved pairing males of the same treatment group (there was no advantage to be gained by pairing males of different treatment groups; see below) and separating the males when they had accumulated 10 s of fighting.

Two males were placed one on each side of a solid wooden barrier that divided a 38 x 29 x 16 cm cage into two equal halves. Starting 4 h later, the barrier was raised and the two mice were allowed to interact. Latency to fight and latency to accumulate 10 s of fighting were recorded. These tests lasted a maximum of 10 min or, as noted above, until a pair had accumulated 10 s of fighting. Each animal was tested only once, and a maximum of 10 pairs was tested on any one day. Previous treatment of the males (running wheel vs. sex testing) was randomized in the experimental design used here. All aggression testing was done blind by a single observer.

The protocol used to assess aggressiveness in females took advantage of the fact that females fight much less frequently and less viciously than males, and it is relatively easy to tell which female attacks and which ultimately submits (3). Thus, there was an advantage here of using a design in which females of different treatment groups were paired, namely determining whether the individuals of one treatment group routinely dominated individuals of another treatment group. Also unlike the situation in males, the relative infrequency and mildness of attacks by females allowed one to obtain finer measurements of aggressiveness (e.g., threatening as well as overt attack).

Ten pairs of females of each possible combination of treatments—control vs. low dose, control vs. high dose, and low vs. high dose—were tested for aggressiveness using the same test arena and timing described earlier for males. Latency to attack, the number of attacks by each member of each pair, and whether the attacked female fought back or ran was recorded. By operational definition, separate attacks occurred a minimum of 3 s apart. Tail rattle, a common indication of impending attack, also was recorded. The definitions of these behaviors are those that have been used for decades in studies of aggressiveness in mice (e.g., 24). All testing was done blind by a single observer (the same one that assessed male aggressiveness).

Autopsy

Three days after the last aggression test, all animals were killed and autopsied. Testes, ovaries, and pituitaries were excised and weighed and the other internal organs were saved for use in other studies.

Statistical Assessment

For reasons noted earlier, separate 1 x 3 ANOVAs were used routinely to analyze the effects of the steroid treatment in each sex. Nonparametric data were analyzed by a 3-cell contingency table. All analyses used Macintosh StatView software.

RESULTS

Reproductive Characteristics

In males, steroid treatment caused a significant decrease in testes weight without any change in the weight of the pituitary (Table 1). Control females showed normal vaginal cycles; that is, all 20 females showed either one or two periods of estrus during the 10-day test period. In contrast, absolutely no cycling was seen in any of the 40 females treated with steroids. Correlatively, steroid treatment caused a significant depression in the weight of both the pituitary and the ovaries in females.

Spontaneous Running

The high dose of steroid treatment depressed the spontaneous use of a running wheel by 44% and 41% for females
Male Sexual Behavior

Even when exposed consecutively to two fully primed females, 8 of 20 individuals ejaculated (p < 0.01) but only 4 of 20 individuals achieved intromission and the longer period of time in which these behaviors could occur was accompanied by a significant depression in ejaculation. The lack of enhancement of male sexual behavior in the steroid-treated males is of particular interest here because it was accompanied by a significant depression in ejaculation. The relevance of the present findings for athletes and body builders depends on which behavior is of interest and the degree to which it is influenced by androgens in humans. The consensus seems to be that androgens do play a regulatory role in the sexual behavior of the human male, acting on libido as well as potency (9). To the degree that the present results with mice are relevant then, they suggest that pharmacological amounts of androgens do not enhance any component of sexual behavior beyond that shown by adult males secreting normal amounts of testosterone. On the other hand, it is well established that aggression and sexual behavior are androgen dependent in male rodents (reviewed by (1)]. The evidence linking human aggressiveness to androgens is weaker (2), but, again to the degree that the present results are relevant, they suggest no increase in aggressiveness over that shown by normal males secreting normal amounts of testosterone.

Finally, of possible importance for humans is the steroid-
induced depression of spontaneous activity that was significant in females but only marginally so in males. As was seen here (Fig. 2), females are normally more active than males, and this is generally true in rodents (19). More germane, it has been known for decades that the use of a running wheel is under the control of estrogen in females [e.g., (13)]. Thus, the depression seen here in steroid-exposed females could reflect simply a decrease in circulating estrogen due to the negative feedback action of androgens on gonadotropin secretion. Certainly the ovaries were greatly reduced in size in the steroid-treated females. On the other hand, if the trend for decreased activity in males is real, albeit not quite significant, then it could indicate a more direct central action of the androgens in both sexes. How this would manifest itself in humans, particularly in relation to athletic performance, is an interesting question.

ACKNOWLEDGEMENT

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### TABLE 2
**LATENCIES TO MOUNT, ACHIEVE INTROMISSION, AND EJACULATE, AND NUMBER OF MALES EJACULATING**

<table>
<thead>
<tr>
<th>Dose of Steroids</th>
<th>Control</th>
<th>Low</th>
<th>High</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to mount (min)</td>
<td>6.1 ± 2.0</td>
<td>6.9 ± 1.0</td>
<td>4.7 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Latency to intromission (min)</td>
<td>32 ± 7</td>
<td>60 ± 13</td>
<td>50 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td>Number mounts, intromissions*</td>
<td>69 ± 13</td>
<td>75 ± 14</td>
<td>96 ± 17</td>
<td>NS</td>
</tr>
<tr>
<td>Latency to ejaculate (min)†</td>
<td>56 ± 5</td>
<td>39 ± 20</td>
<td>75 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>Number ejaculating</td>
<td>8/10</td>
<td>2/10</td>
<td>2/10</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are mean ± SE. N = 10 in each group.

*The total number of mounts and intromissions occurring between the initiation of pairing and either ejaculation or the end of the 2 h test.

†Calculated only on the basis of the males that ejaculated.

### TABLE 3
**NUMBER OF PAIRS OF MALES IN WHICH FIGHTING OCCURRED, LATENCY TO THE FIRST FIGHT, AND LATENCY TO ACCUMULATE 10 s OF FIGHTING**

<table>
<thead>
<tr>
<th>Dose of Steroids</th>
<th>Control</th>
<th>Low</th>
<th>High</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of pairs fighting</td>
<td>9/10</td>
<td>9/10</td>
<td>9/9</td>
<td>NS</td>
</tr>
<tr>
<td>Latency to first fight (s)</td>
<td>212 ± 48</td>
<td>240 ± 50</td>
<td>229 ± 50</td>
<td>NS</td>
</tr>
<tr>
<td>Latency to 10-sec fighting (s)</td>
<td>289 ± 46</td>
<td>341 ± 63</td>
<td>326 ± 60</td>
<td>NS</td>
</tr>
</tbody>
</table>

N = 10 pairs in each group.

### TABLE 4
**NUMBER OF THREATS (TAIL RATTLING) AND ATTACKS INITIATED BY FEMALES OF THE THREE TREATMENT GROUPS, THE PERCENTAGE OF THESE ATTACKS IN WHICH THE ATTACKED MOUSE FOUGHT BACK (AS OPPOSED TO RUNNING AWAY), AND THE MEAN NUMBER OF NONRECEPTIVE RESPONSES SEEN**

<table>
<thead>
<tr>
<th>Dose of Steroids</th>
<th>Control</th>
<th>Low</th>
<th>High</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instances of tail rattling</td>
<td>0.2 ± 0.2</td>
<td>3.1 ± 1.3</td>
<td>3.0 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Number of attacks initiated</td>
<td>0</td>
<td>3.7 ± 1.1</td>
<td>2.5 ± 0.8</td>
<td>0.005</td>
</tr>
<tr>
<td>Proportion of attacks fought back*</td>
<td>6 ± 6</td>
<td>56 ± 14</td>
<td>58 ± 18</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Values are mean ± SE. The first two comparisons are based only on the pairings in which fighting occurred (tail rattling was never seen in pairings in which attack behavior was absent). N = 10 pairs in each group.

*The percent of times that an attacked female fought back rather than running away.
REFERENCES