Behavioural Anxiolytic Effects of Low-Dose Anabolic Androgenic Steroid Treatment in Rats

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ÅGREN, G., I. THIBLIN, P. TIRASSA, T. LUNDEBERG AND C. STENFORS. Behavioural anxiolytic effects of low-dose anabolic androgenic steroid treatment in rats. PHYSIOL BEHAV 66(3) 503–509, 1999.—The use of anabolic androgenic steroids (AAS) in supratherapeutic doses has been associated with aggressive behaviour as well as with severe affective and psychotic symptoms. These symptoms usually follow a chronic exposure for several months. However, AAS also may have milder effects with hypomania-like features such as an increase in confidence, energy and self-esteem. We have studied the short-term effects on male rat behaviour in a modified open-field test of the AAS Metenolon administered three times at a low dose (0.01 mg/kg/week × 3). The control rats showed indications of increased timidity and aversive learning following retesting, a reaction that was absent in the AAS-treated rats. The AAS-treated rats showed less fear or anticipatory anxiety compared to control animals. Furthermore, the suppressed marking behaviour and altered morphological allometric relationships were compatible with a modified social and sexual competence in the AAS treated rats. © 1999 Elsevier Science Inc.

Anabolic–androgenic steroids Metenolon Open field Exploration Anxiolysis Endocrine glands

A substantial body of information has been accumulated, suggesting that the use of anabolic–androgenic steroids (AAS) in supratherapeutic doses may result in behavioural disturbances, including an increased level of aggression (2,3,9,11,18,19). These symptoms are usually seen in persons who have used AAS for several months. However, early psychological effects have been described as mania-like, featuring symptoms such as euphoria, increased confidence, energy, and self-esteem (2,15,19). Although the primary reason for using AAS usually is to enhance athletic performance or physical appearance, the above psychological effects are also considered desirable (13,14,17,19). Such effects could be compatible with the behaviour observed in rats which, following 6 days of exposure to high levels of testosterone propionate (3.5–5 mg/kg/day), showed reduced levels of anxiety as measured in an elevated plus-maze test (4). In the present study we are investigating if repeated weekly doses of an AAS (0.01 mg/kg/week × 3), Metenolon (17β-hydroxy-1-methyl-5α-androst-3-one) may lead to behavioural changes reflecting reduced anxiety in male rats. Thus, the influence of this low-dose Metenolon treatment on a number of behaviour patterns was studied in a modified open-field test (1), where the rats had access to not only a closed and an open area similar to the plus-maze test (4,12), but additionally, to a brightly illuminated area. The rats were directly observed, and several behavioural parameters were registered to identify the emotional states expressed.

Data on body weight, growth, and weights of preputial, adrenal, and reproductive glands were collected to investigate the presence of anabolic–androgenic effects of the dose regimen applied. Furthermore, the presence of anxiolytic effects of the AAS treatment was examined on chronic, rank-related social stress effects due to social housing, as reflected by the variation in gland size and in scent-marking behaviour. The level of anxiety can be due to novel environmental stimuli, as found in the open-field situation, or to social stimuli from a dominant group member in the home cage (8). The “chronic” challenge due to social housing can be expected to affect morphological traits, but not the temporary challenge in the open field test.

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METHODS

Animals and Housing

Twenty male Sprague–Dawley rats (250–300 g) (B&K Universal AB, Sollentuna) were kept in permanent groups of five per cage, which may cause chronic social stress (8). Ten rats were randomly assigned to the control group, and 10 to the experimental group. The rats in each cage received the same treatment. They were housed on a 12 L:12 D cycle, lights on at 0700 h, and provided standard rat food and water ad lib. The tests took place in the late afternoon in the same room where the rats were housed. Eight female rats were housed in this room about one meter from the testing arena.

Drug Treatments and Testing Schedule

The rats were tested once before receiving the first drug treatment. They were then given intraperitoneal injections (i.p.) with Metenolon (17β-hydroxy-1-methyl-5α-androstane-3-one; 0.01 mg/kg) or the vehicle ricinoleic acid on the first day of 3 consecutive weeks. The rats were subsequently tested on Day 4 following the injections. Thus, all rats were tested four times with an interval of 1 week. The choice of substance, and dose regimen, respectively, was based on an unpublished pilot study. The results indicated that the dose regimen used here caused a pronounced change in several neurotransmitter concentrations in brain regions that are linked to regulation of mood, alertness, and emotion, while no peripheral anabolic–androgen effects were found.

The Testing Arena and Behavioural Parameters

The testing arena was a 150 × 100-cm “open field,” which had no cover, except for the starting box. The arena was divided in one shaded half and one well illuminated (100 W) half separated by a 5-cm barrier. An opaque 22 × 17 × 12-cm plastic starting box was placed in the corner between the wall and the 5-cm barrier in the shaded half. The 5 × 10-cm opening was facing away from the illuminated half. The shaded half of the arena was subdivided in four areas: the opaque plastic starting box [0], a 12-cm wide zone along the wall near the starting box [1], a 12-cm wide zone along the opposite wall from the box [2], and the central area [3]. Area 3 was closer to the starting box than area 2, although without close proximity to walls. Half of the 12-cm wide zone along the 100-cm wall between areas 1 and 2 was included in area 1, and half in area 2. The entire well-illuminated half of the open field on the opposite side of the barrier was considered one area [4].

To allow the rats to become familiar with the starting box, it was placed for 30 min in the communal home cage of each group of rats to be tested consecutively, and soiled sawdust from that home cage was put in the starting box. The box with one rat was next placed in position in the open field, and the rat was prevented from leaving for 30 s before being allowed free movement. Each rat was observed directly for 10 min. Five parameters were recorded: 1) the latency of the first entry into each area, 2) the cumulative time spent in each area, 3) the number of area changes as a measure of ambulation, 4) the rearing frequency, and 5) urine marking, i.e., the number of small urine pools counted after the test of each rat. An entry was registered if the rat’s four feet were inside the zone in question. The entry latency to area 1 is equivalent to the emergence latency out of the starting box.

Body and Endocrine Gland Weights

The body weight of each rat was registered daily. After decapitation on Day 1 of the week following the final testing day, the weight of the rats’ heart, adrenal, and preputial glands, testes, vesiculae seminales, and epididymides were assessed.

Statistical Analysis

The Kruskal–Wallis ANOVA was used to analyse variance between tests, followed by Wilcoxon matched-pairs signed-ranks test. For comparison between treatment groups the Mann–Whitney U-test was used. Linear regression was used for body and gland weight data following LOG transformation of the data. For correlations including behavioural parameters, Spearman’s rank test was used. Factor analyses were performed on 1) data collected before the injections, 2) data regarding the vehicle-injected rats, and 3) the AAS-treated rats, respectively, to reduce the number of variables and facilitate the interpretation of the data.

RESULTS

Pre-drug Treatment Behaviour

The behaviour patterns of the control and experimental rats were not statistically different with regard to time spent, entry latency, entry frequency, rearing, or urine marking on the first testing occasion performed when the rats were naive to the area (Figs. 1, 2, and 3). The rats spent 60% of the testing time or more in the starting box on this first testing occasion. Four control and four experimental rats spent 3% of the time or less in the well-illuminated area 4 (Fig. 1). The highly variable emergence latencies from the starting box into zone 1 was, on average, about 100 s (Fig 2).

FIG. 1. Proportion time allocated to each open-field area. The subdivision of the bars represent from bottom to top the closed area 0, and the open areas 1 to 4. The statistics in the figure refer to comparison between vehicle and Metenolon-treated rats for each area and testing occasion (Mann–Whitney U-test: *p < 0.05, **p < 0.01, ***p < 0.001).
The Behaviour of the Vehicle-Treated Control Rats Following Repeated Testing

Time. The cumulative time spent in the starting box increased (Fig. 1; ANOVA, \( \chi^2 = 13.5, p < 0.004 \)) on the testing occasion following the first injection (\( p < 0.01 \)). Then, the rats reduced time spent in areas 1 (Fig. 1: \( \chi^2 = 13.6, p < 0.004 \)) and 3 (\( \chi^2 = 7.9, p < 0.05 \)), a reduction significant following the first injection (\( p < 0.01 \) and \( p < 0.02 \)). Only one rat visited the illuminated area 4 once in the tests after the vehicle injections.

Latencies. The emergence latency out of the starting box increased (Fig. 2: ANOVA; \( \chi^2 = 18.1, p < 0.0004 \)) following the first injection on the second (\( p < 0.01 \)), compared to the previous testing occasion. The latency of leaving the box into area 1 remained prolonged on the following testing occasions. The entry latencies to area 2 and 3 did not change significantly. The increased latency, or failure, to enter area 4 in the tests following the injections was significant (ANOVA; \( \chi^2 = 8.6, p < 0.04 \)).

Ambulation. The ambulation score, as well as the entry frequency to each separate area was significantly reduced (Fig. 3a; ANOVA; \( \chi^2 = 7.6 \) to 13.2, \( p = 0.055-0.004 \)). Again, the significant reduction occurred following the first injection (\( p < 0.05 \)).

Rearing. Similarly, rearing frequencies per 10 min decreased significantly (Fig. 3b: \( \chi^2 = 10.3, p < 0.02 \)) on the first testing occasion following the first vehicle injection compared to the previous testing occasion, although the rearing frequency per minute when out of the starting box increased throughout the study (\( \chi^2 = 12.7, p = 0.005 \)).

Urine marking. Not all males marked; during the three postinjection tests three, two, and three rats did, respectively. The individual range across all tests was 0–13 (Fig. 3c).

The Behaviour of the Metenolon-Treated Rats Following Repeated Testing

Time. The AAS-treated rats did not significantly change the time spent in the starting box, the peripheral [1] or central [3] areas with increasing number of AAS injections, although the time spent in the starting box [0] tended to decrease following the second injection (Fig. 1). However, they allocated significantly more time to the far peripheral area 2 (Fig. 1: ANOVA, \( \chi^2 = 8.4, p < 0.02 \)) after three treatments (\( p < 0.05 \)) and to the illuminated area 4 (\( \chi^2 = 9.8, p < 0.02 \)) following two drug treatments (0.06).

Compared to control rats, the steroid-treated rats spent significantly less time in the starting box, and significantly
more time in all open-field areas after 1 and/or 2, and 3 weeks of drug treatment, as indicated in Fig. 1 (p = 0.06–0.001).

Latencies. All area entry latencies tended to decrease following the second AAS injection, but not significantly so (Fig. 2). Compared to the latencies of the control rats, however, the latencies of the Metenolon-treated rats were significantly shorter following the second and third AAS treatment, as indicated in Fig. 2 (p < 0.05–0.01).

Ambulation. The sum of entry frequencies, that is, the ambulation score in the AAS-treated rats, increased after two injections (Fig. 3a; ANOVA; χ² = 9.7, p < 0.02). Ambulation scores were significantly higher in the AAS-treated rats compared to controls after 2 weeks of drug treatment (Fig. 3A; p < 0.05). The increase was specifically due to an increased number of entries into areas 2 (χ² = 8.2, p = 0.04) and 4 (χ² = 8.4, p < 0.04). The number of entries into the other zones did not change significantly.

Rearing. The AAS-treated rats’ scores did not vary significantly among tests, but the AAS-treated rats reared more within the 10-min tests than the vehicle-injected rats did after the first and second injections (Fig. 3B). However, following three injections the control rats reared significantly more often per minute than the AAS-treated rats when out of the starting box (not in Fig. 3B; p < 0.01).

Urine marking. The AAS-treated rats rarely deposited any urine drops. One of the 10 rats urinated marked once during the first and once during the second post-injection tests (Fig. 3C). On the last testing occasion none did.

Factor Analyses

Untreated naive rats. The behaviour of the experimental and control rats did not differ on the first testing occasion before the drug injections (Figs. 1–3). Data from the two groups were, therefore, pooled.

Three factors with Eigenvalues above 1 explained 84% of the variation in the variables describing ambulation and rearing, entry latencies, and time spent in each area (Table 1). The latencies of entering area 1, 2, and 3 showed strong negative loadings on the first factor. The entry latencies also showed negative loadings on the second factor, but only to areas 2, 3, and 4. Time spent in these same areas, ambulation, and rearing showed positive loadings on both factors. The entry latency into the illuminated area 4 only showed a positive strong loading on the third factor.

Vehicle-treated rats. The factor analysis of the data regarding the three tests after the vehicle injections also indicated that three major factors with Eigenvalues above 1 explained a high proportion, or 85% of the variation in the data. However, time spent in zone 4 and latencies of entering zone 4 were largely missing (see Fig. 2).

The various parameters showed similar loadings on the first factor (Table 1) as found in the untreated rats, except the entry latency to central area 3. The entry latency to the central area 3 showed a positive loading on the second factor. The number of tests and/or AAS injections received showed a strong load on the third factor.

AAS-treated rats. Again, three factors with Eigenvalues above 1 explained most, or 80% of the variation (Table 1). Similar loadings on the first factor were found as in the two previous analyses (Table 1). However, similar to the first pre-injection test, time spent in areas 3 and 4 showed strong positive loadings, and time spent in the starting box negative loadings on the second factor. The entry latencies to the illuminated area 4, and the number of tests and/or AAS injections received showed negative loadings on the second and third factors, respectively, unlike the vehicle-treated rats.

Body Size and Behaviour Correlations

Growth and gland sizes. Neither body size nor growth rates before or after the drug treatments were different in the control and experimental groups (Table 2). Nor was the weight of their heart, adrenals, testes, vesiculae seminales, and epididymides or their preputial glands, respectively, sig-
LOW-DOSE AAS-INDUCED ANXIOLYSIS IN RATS

TABLE 1

VARIMAX ROTATED FACTOR LOADINGS FOR BEHAVIOUR OBSERVED IN A MODIFIED OPEN-FIELD TEST

<table>
<thead>
<tr>
<th>Factors</th>
<th>A. Untreated Rats</th>
<th>B. Vehicle-Treated Rats</th>
<th>C. AAS-Treated Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test*</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Time 0</td>
<td>0.74</td>
<td>-0.56</td>
<td>0.19</td>
</tr>
<tr>
<td>Time 1</td>
<td>0.80</td>
<td>0.15</td>
<td>-0.24</td>
</tr>
<tr>
<td>Time 2</td>
<td>0.55</td>
<td>0.71</td>
<td>-0.05</td>
</tr>
<tr>
<td>Time 3</td>
<td>0.60</td>
<td>0.67</td>
<td>-0.12</td>
</tr>
<tr>
<td>Time 4</td>
<td>0.07</td>
<td>0.94</td>
<td>-0.19</td>
</tr>
<tr>
<td>Latency 1</td>
<td>-0.75</td>
<td>0.00</td>
<td>0.36</td>
</tr>
<tr>
<td>Latency 2</td>
<td>-0.84</td>
<td>-0.33</td>
<td>0.05</td>
</tr>
<tr>
<td>Latency 3</td>
<td>-0.84</td>
<td>-0.34</td>
<td>0.05</td>
</tr>
<tr>
<td>Latency 4</td>
<td>-0.12</td>
<td>-0.15</td>
<td>0.95</td>
</tr>
<tr>
<td>Ambulation</td>
<td>0.82</td>
<td>0.54</td>
<td>-0.05</td>
</tr>
<tr>
<td>Rearing</td>
<td>0.87</td>
<td>0.26</td>
<td>0.10</td>
</tr>
<tr>
<td>Eigenvalues:</td>
<td>7.1</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Variation, %</td>
<td>64%</td>
<td>10%</td>
<td>9%</td>
</tr>
</tbody>
</table>

*Test = testing occasion. Time 0 = time spent in the closed starting box, and Time 1 to 4 = time spent in each open area. Latency 1 to 4 = entry latencies into each area.

Behaviour and weight correlations. The rearing (ri = 0.68, p < 0.04), ambulation (ri = 0.80, p < 0.02), and urine marking frequencies (ri = 0.80, p < 0.02), respectively, of the vehicle-treated control rats during the final test, correlated positively with the combined weight of their vesiculae seminales (VS) plus epididymides (E). Furthermore, the time they spent in the starting box (ri = -0.80, p < 0.02) and emergence latencies out of this box (ri = -0.63, p < 0.06), respectively, correlated negatively with the weight of their VS + E. The corresponding relationships were statistically insignificant in the AAS-treated rats.

DISCUSSION

The data obtained from the open-field test demonstrate clear-cut differences in the behaviour of vehicle and Metenolon-treated rats, which suggest an anxiolytic drug effect, while no pronounced anabolic effects were found. Yet, the relationships between behaviour, body, and endocrine gland weight suggest that not only the stress response to novel environmental, but also social stimuli were modified. The behavioural effects were found at a dose that did not induce anabolic effects.

The vehicle-treated rats showed an increased avoidance of the open space and preference for the closed starting box on retesting 1 week after the initial experience of the testing situation. This new behaviour prevailed on the two subsequent testing occasions. A similar change in behaviour is observed following retesting in plus-maze anxiety tests (12). The opposite reaction to retesting was found in the AAS-treated rats. These rats rather increased time spent out of the box, particularly in distant areas, including the illuminated part of the open field. Because the control rats reduced the time spent out of the box, they also reduced the amount of ambulation.

TABLE 2

WEIGHT AND GROWTH DATA

<table>
<thead>
<tr>
<th></th>
<th>Body Weight (g)</th>
<th>Daily Growth (g)</th>
<th>Heart Dry Weight (mg)</th>
<th>Adrenal Weight (mg)</th>
<th>Testes Dry Weight (mg)</th>
<th>Vesic SEM* + Epididymis (mg)</th>
<th>Preputial Gland Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>mean 366</td>
<td>4.8</td>
<td>252</td>
<td>58</td>
<td>494</td>
<td>1420</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>SD 20</td>
<td>1.4</td>
<td>12</td>
<td>6</td>
<td>39</td>
<td>210</td>
<td>21</td>
</tr>
<tr>
<td>AAS</td>
<td>mean 366</td>
<td>4.4</td>
<td>254</td>
<td>58</td>
<td>481</td>
<td>1570</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td>SD 24</td>
<td>1.5</td>
<td>15</td>
<td>9</td>
<td>39</td>
<td>250</td>
<td>38</td>
</tr>
</tbody>
</table>

*Vesiculae seminales.
and rearing following the vehicle treatment, while the AAS-treated rats showed more ambulation and more rearing within the 10-min test period. Some behavioural differences were found following one injection, but the differences were clearly established following the second weekly drug injection. Previously, a similar anxiety-like effect has been observed in a plus-maze test only after 6 days of chronic high-dose testosterone propionate (3.5–5 mg/kg/day) exposure (4).

The AAS-treated rats' growth rate was not different from that of the control rats, nor was the average size of their adrenal or sexual glands. Although the weight of the preputial glands was similar, the testosterone-dependent glandular activities of the AAS-treated rats were reduced, as judged visually by the colour and structure of the glands. Furthermore, one or two rats per cage among the vehicle-treated rats urinemarked, a social rank-related behaviour that was lost in all AAS-treated rats. The number of urine drops found after each test correlated with the tested rats' vesiculae seminales plus epididymides. In addition, the weight of the control rats' preputial glands and their vesiculae seminales plus epididymides were significantly correlated, indicating the release of sexual signalling of both urine marking and the preputial gland secretion (6).

The allometric relationship found between the vehicle-treated rats' body and heart weights, but not their adrenal and sexual glands, suggests that the size of the endocrine glands was affected by psychosocial stress, reflecting the differential reproductive and social status normal within groups housed in the same cage (8). However, unlike the vehicle-treated rats, allometric relationships were found in the AAS-treated rats between their body weight, and adrenal, and testes, respectively, suggesting the differentiating impact of psychosocial stress on gland size was lost following the AAS treatment. On the other hand, body and heart weights of the AAS-treated rats were not correlated, possibly reflecting a beginning anabolic effect.

Because conflicting drives can explain the behaviour observed in a novel environment (1,5,16), the interpretation of the data is not straightforward. For example, ambulation and rearing can reflect flight, or it may be expression of exploratory behaviour. The repeated testing will increase experience and promote learning. This can induce anticipatory anxiety (7), or it may reduce anxiety (5,16). Exploratory behaviour can be sexually motivated or general. The above positive correlations found between the sperm stores of the vehicle-treated rats and their ambulation, rearing, and urine marking frequencies, and negative correlation to the time they spent inside the starting box support the idea of an impact of different sexual motivation on these rats' exploratory behaviour, likely reinforced by the presence of females in the room.

Approximately 60% of the variation in the data was expressed in the first factor whether or not the rats had received any drug treatment or had previous experience of the open field. In view of the strong loading of ambulation and rearing on the first factor under all conditions, this factor is interpreted as an exploratory drive.

Delayed emergence, low ambulation scores, avoidance of brightly lit, open, or central areas away from walls in open fields have previously been found to reflect "emotionality" or fear in rats and mice in the plus-maze and open-field tests (1,12). Because the starting box in this study was, to some extent, "familiarised" by soiled wood shavings from the home cage, and the cover provided some protection, it is likely that the rats developed a sense of security inside the box. Therefore, extended emergence latencies from the box should reflect timidity. The loadings on the second factor by the different variables could be compatible with escape in the naïve rats. In contrast, in the retested vehicle-treated rats the second factor is rather consistent with anxiety and withdrawal, considering 1) the delayed emergence from the box, 2) a delayed entry into the central area, and 3) total avoidance of the illuminated part of the open field. The effect is likely reinforced by aversive learning during the first testing occasion as observed in plus-maze testing (12). Because opposite loadings were found in the AAS-treated rats, an opposite reaction was indicated in these rats. Assuming that the second factor still reflects the level of anxiety, timidity, or vigilance, the AAS-treated rats grow less timid or vigilant.

The third factor could reflect aversive learning (12), considering the strong positive load on the consecutive occasion of testing and/or injection in the vehicle-treated rats, and strong negative load in the AAS-treated rats. The vehicle-treated rats did not enter the illuminated area, whereas a strong load on the latencies of entering this area was found in the naïve rats.

This suggests stimuli from this area, for example, the light was aversive. The strong negative load in the AAS-treated rats on the number of tests and injections suggests they did not develop anticipatory anxiety or fear, that is, they did not learn.

In conclusion, the behaviour of the vehicle-treated rats can be interpreted as a passive stress response with withdrawal, possibly due to first trial aversive learning (12), while the AAS-treated rats rather reacted actively without restraint. The behaviour following the administration of Metenolon is compatible with reduced vigilance, fear and/or anticipatory anxiety, which would require aversive learning. In parallel, morphological indices implicate a suppressed sexual and social competence. The behavioural effects in the rats may correspond to a change in mood, for example, with increased confidence and self-esteem, but also cognitive impairment, reported to occur as an early effect of AAS administration in humans (15,17,19).

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REFERENCES

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