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EFFECTS OF CHRONIC TREATMENT WITH TESTOSTERONE PROPIONATE ON AGGRESSION AND HORMONAL LEVELS IN INTACT MALE MICE

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SUMMARY

Effects of testosterone propionate, an anabolic-androgenic steroid (AAS), on aggression in gonadally intact male mice were examined. Animals were given weekly injections of 3.75, 7.5, 15, and 30 mg/kg of drug or sesame oil for 10 weeks. During the last 3 weeks, behavioral tests were conducted and at the end of the experiment, body, liver and testes weight and hormonal data were collected. The treatment had minimal behavioral and endocrine effects. It resulted in shorter latencies of 'threat' only in the last agonistic encounter, increases in testosterone levels and decreases in testes weight in a non-linear dose-dependant way. The action of the treatment was different on threat and attack, the latter being unaffected. The behavioral effects in the total sample were only found in aggressive animals selected on the basis of their latency of attack in the first encounter.

Keywords—AAS; Testosterone; Corticosterone; Aggression; Intact male mice; Individual differences.

INTRODUCTION

As a consequence of anabolic-androgenic steroid (AAS) abuse, side effects on behavior such as altered libido, changes in mood, psychotic episodes, increased irritability and aggressive behavior have been described, with these latter effects being the most commonly reported by users (Bond et al., 1995; Choi, 1993; Choi et al., 1989; Conacher and Workman, 1989; Dalby, 1992; Hannan et al., 1991; Pope and Katz, 1994; Salvador et al., 1994; Su et al., 1993; Uzych, 1992; Wandler, 1994). In fact, violent assault, attempted murder and, in extreme cases, homicide have been said to be committed under AAS influence (Conacher and Workman, 1989; Pope and Katz, 1990). The wide acceptance of the aggression-facilitating power of the AAS has led to the use of a legal defense strategy called the 'Dumbell strategy' in trials (Bahrke et al., 1990; Choi, 1993). AAS are abused by sportsmen in competitive and non-competitive sports (Lamb, 1984; Tricker et al., 1989; Yesalis et al., 1993), although, lately, adolescents have begun to take them to enhance their physical

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appearance and strength in a short period of time (Du Rant et al., 1993, 1995; Komoroski and Rickert, 1992; Korkia and Stimson, 1994; Middleman et al., 1995; Morrison, 1994; Williamson, 1993).

Despite the general social acceptance that AAS have a facilitating effect on aggression, there is not sufficient experimental proof to support this since only few studies have been published on this topic in humans and animals. In view of the social impact of the high incidence of AAS abuse as well as the associated risk of antisocial behavior, experimental studies are necessary to determine if there is an association between these substances and aggression (Svare, 1990). The use of gonadally intact animals, to mimic what happens in human abusers, and of well established animal models in the study of the biological basis of aggression permits this experimental approach. A few studies, using different strategies to analyze the effects of specific AAS on aggression in non-castrated male rodents have recently been published. Using a model of competition for food, Bonson and Winter (1992) and Bonson et al. (1994) reported that a 2 week administration of 15 mg/kg per day of testosterone propionate (TP) induced dominance in rats which were previously non-dominant. Lumia et al. (1994), in rats, using a model of isolation-induced aggression, found that the administration of 15 mg/kg per week of TP for 10 weeks increased the number of threats and dominant postures in the last 2 weeks of treatment. However, Sandnabba et al. (1994) injecting 25 mg/kg per day for 1 week found no differences in a 7-point rating aggression scale in male mice. Lately, researchers using a 'cocktail' of several AAS started to study physiological (Ahima and Harlan, 1992; Clark et al., 1995; Menard et al., 1995) and behavioral effects, and found no effects on aggression in gonadally intact male mice after a combination of four AAS at several doses for 6 months (Bronson, 1996).

In a number of previous studies, we analyzed the effects of AAS on agonistic behavior in mice, finding that different doses, substances and duration of treatment in addition to individual features have different, often contradictory, impacts on offensive behavior in gonadally intact male mice. A single administration of a wide range of doses of TP, nandrolone decanoate (ND), and a mixture of both subtances resulted in slight increments of threat in those animals which received 3.75 or 30 mg/kg of TP (Moya-Albiol et al., 1995), decreased attacks in those injected with 15 mg/kg of ND (Moya-Albiol et al., 1996) and had no effect on animals treated with the mixture (Moya-Albiol et al., 1997). Chronic administration of stanozolol (0.07, 0.7 and 7 mg/kg) for 3 weeks had different effects on aggression depending on the age of subjects. In young mice there was an increase in aggressive behavior while this pattern was inverse in older animals, although none of the changes were statistically significant (Martínez-Sanchis et al., 1996).

The aim of the present study was to elucidate the influence of AAS on aggressive behavior. Thus, the effects of a long term administration (10 weeks) of TP (a long acting testosterone ester and one of the more abused AAS) on agonistic behavior in intact male mice were evaluated, using a model of isolation-induced aggression that is frequently employed to assess the action of psychoactive substances on social interaction in mice. The doses employed (3.75, 7.5, 15 and 30 mg/kg per week) mimic a moderate–high range of abuse in humans and fluctuate between 3- and 30-fold higher than the maximum dose employed for therapeutic purposes. At the end of the experiment, blood samples were taken to study the relationship between changes in aggression and hormonal levels resulting from the treatment. Finally, body, liver and testes weights were recorded in order to evaluate the anabolic and androgenic effects of TP.

METHODS

Subjects

One-hundred OF1 male mice, from Iffa Credo (France), aged 42 days, were individually housed in plastic cages (20 × 10 × 13 cm) and used as experimental animals. A further 290 animals were housed in groups of five in larger cages (21.5 × 21.5 × 15 cm) and used once as 'standard' opponents, after being rendered temporally anosmic by intranasal lavage with a 4% zinc sulphate solution a day before testing. Anosmic mice were employed as standard opponents because they elicit attack but never initiate such behavior (Parmigiani and Brain, 1983). All animals were subjected to a 12-h light/dark cycle (lights on 1900–0700h). Laboratory temperature was maintained at 20 and 22°C. Food and water were supplied ad libitum.

Drug

Testosterone propionate (TP) (commercial name Testex Leo®; Leo S.A., Madrid, Spain) was dissolved in adequate sesame oil volumes to provide the following doses: 3.75, 7.5, 15 and 30 mg/kg and was administered weekly for 10 weeks. Sesame oil was injected in controls. Drug or vehicle was injected intramuscularly in a volume of 0.1 ml.

Procedure

After an isolation period of 3 weeks, experimental mice were randomly allocated to five different groups; each receiving one of the TP doses or sesame oil. Individual animals were always injected the same day of the week and weighed once a week during the treatment period.

During the last 3 weeks of treatment, behavioral tests were carried out 24 h after the injection. Twenty-four hours after the last behavioral test, experimental animals were given a lethal dose of the barbiturate Tiopental (Tiobarbital®; Palex S.A., Jaén, Spain) by intraperitoneal injection. Blood samples were collected and testes and liver were removed and weighed.

Social Encounter test

Experimental animals confronted anosmic opponents for 10 min in a neutral area (59 × 29 × 32.5 transparent glass cage) illuminated by a red light (25 W). Social encounters were preceded by a minute of adaptation in which the animals were separated by a plastic partition. The behavioral tests started in the 2nd h of the subjects' dark period and were recorded with a video camera positioned in front of the test cage.

The behavior of the experimental animals was evaluated using an ethological technique based on a computerized observational procedure which was developed by Brain et al. (1989). The behaviors are classified in 11 broad categories. Each category includes a variety of different behavioral postures and elements. The categories and their constituent elements are as follows: (1) body care (abbreviated groom, self-groom, wash, shake, scratch); (2) digging (dig, kick dig, push dig); (3) non-social exploration (explore, rear, supported rear, scan); (4) exploration from a distance (approach, attend, circle, head orient, stretched attention); (5) social investigation (crawl over, crawl under, follow, groom, head groom, investigate, nose sniff, sniff, push past, walk around); (6) threat (aggressive groom, sideways offensive, upright offensive, tail rattle); (7) attack (charge,

lunge, attack, chase); (8) avoidance/flee (evade, flinch, retreat, ricochet, wheel, startle, jump, leave, wall clutch); (9) defensive/submissive (upright defensive, upright submissive, sideways defensive); (10) sexual behavior (attempted mount, mount); (11) immobility (squat, cringe).

A detailed description of all elements can be found in Martínez et al. (1986) and Brain et al. (1989). The analysis of the videotapes involved assessment of the behavior of only the experimental animals. This analysis was carried out by a trained observer who was blind to the experimental group to which each animal belonged. The computer program gives information of total duration (accumulated time spent in each category), frequencies (number of occurrences of each category in the 10 min test) and latency of each category.

No avoidance/flee, defensive/submissive, sexual behavior and immobility were recorded, so these categories do not appear in the results.

Hormone Assay

Blood was withdrawn by cardiac puncture from animals which were injected with a lethal thiobarbital injection 24 h after the last behavioral test. Serum samples were prepared by centrifugation and stored at $-20°C$ until assay. Serum testosterone and corticosterone analysis of the blood samples were carried out by Montoro SCL (Valencia, Spain) using Diagnostic Products Radioimmunoassay Test Kits for testosterone and corticosterone (Diagnostic Products Corporation, LA). The commercial kit used for testosterone determination was Coat-A-Count Total Testosterone and the sensitivity was 4 ng/l. The intra and interassay variation coefficients were 7.5 and 6.9%, respectively. Corticosterone was determined using Coat-A-Count rat corticosterone and the sensitivity was 5.7 ng/ml. The intra and interassay variation coeficients were 4.3 and 5.8%, respectively. Testosterone and corticosterone values were the mean of duplicate determinations and were expressed as ng/ml. In order to have an appropriate control for hormonal levels, data from isolated and grouped animals of the same strain and similar age were recorded following the preceding procedure.

Statistical Analyses

When distributional criteria were met, parametric statistics (repeated measures or one-way ANOVAs for five group comparisons and Newmann-Keuls tests for two group comparisons) were used. However, when data had non-normal distributions, as in the case of the latency of attack and threat, Kruskal-Wallis tests and Mann-Whitney U tests for two group comparisons were carried out. In order to correlate different variables Spearman or Pearson correlations were used depending on the data. Values of $p \leq .05$, two-tailed, were considered significant.

RESULTS

Behavioral Categories

When the evolution of the offensive categories (threat and attack) over weeks was analyzed, some differences were found. Fig. 1 shows total duration, frequency and latency of threat. Total duration of threat was significantly reduced with time ($F_{2,120} = 10.62$; $p < .00$), although neither the 'treatment' nor the 'treatment $\times$ time' interaction was

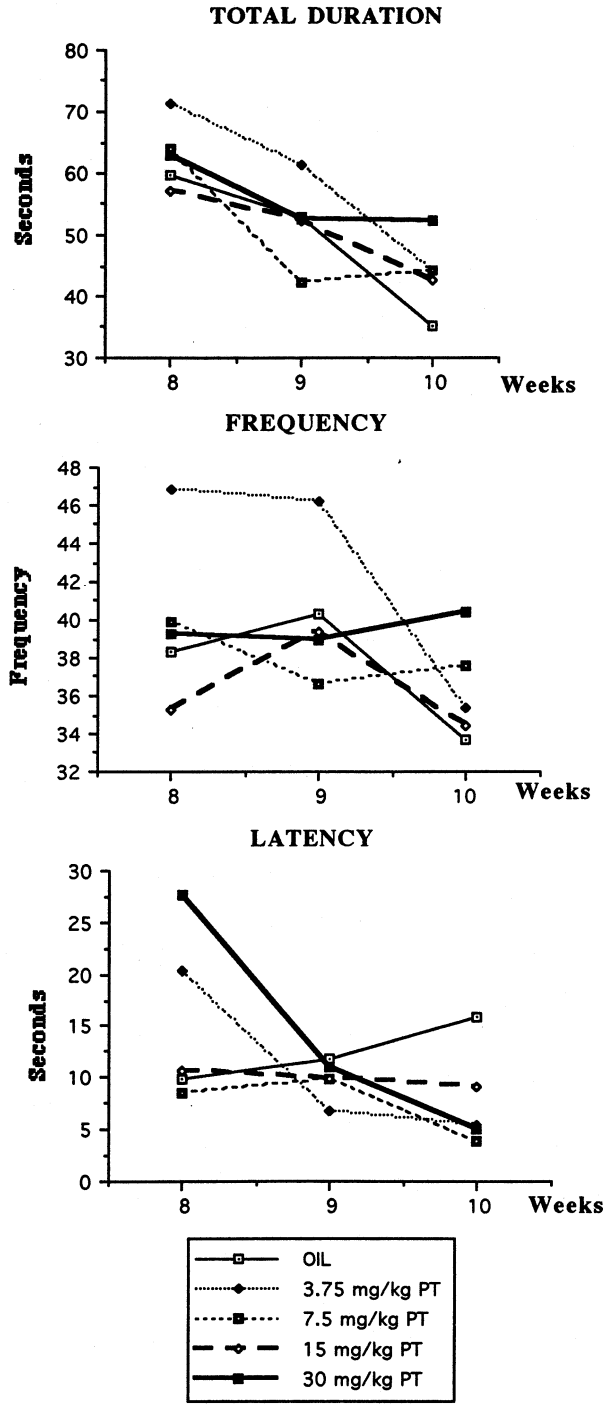

Fig. 1. Total duration (in seconds), frequency and latency (in seconds) of threat category displayed by TP-treated animals and controls on the 8th, 9th and 10th weeks of treatment.

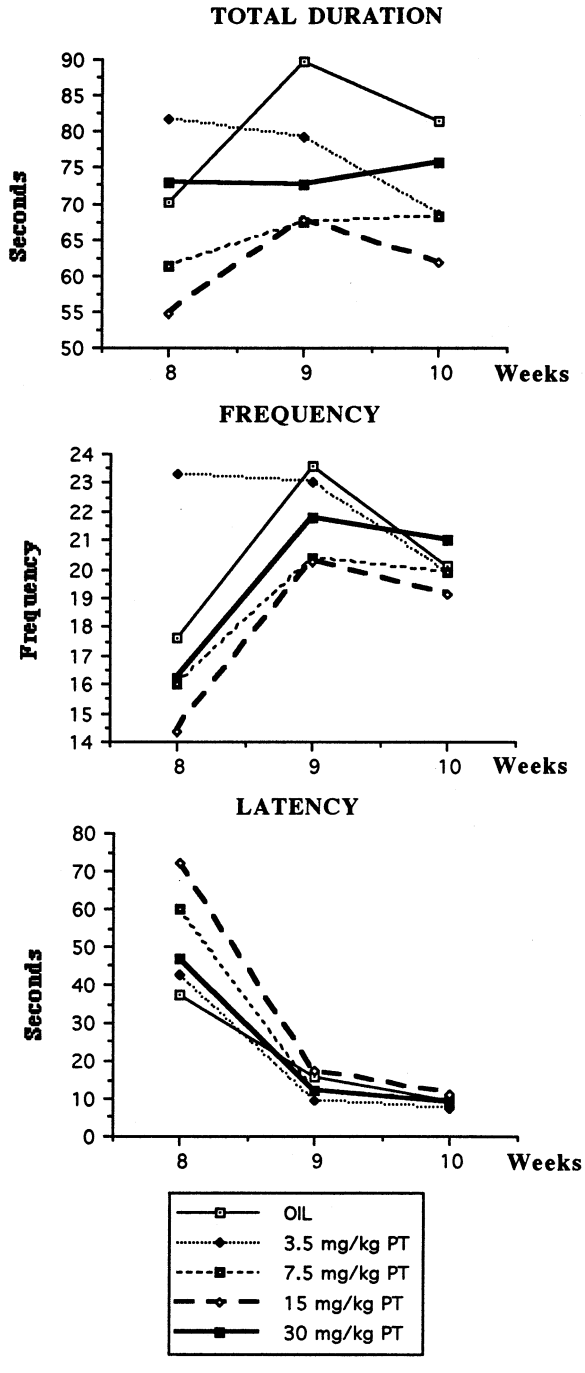

Fig. 2. Total duration (in seconds), frequency and latency (in seconds) of attack category displayed by TP-treated animals and controls on the 8th, 9th and 10th weeks of treatment.

Table 1. Total duration (in seconds) of behavioral categories displayed by TP-treated animals and controls on the 8th, 9th and 10th weeks of treatment

		Sesame oil			Treatment (mg/kg)											
			3.75			7.5			15			30				
		Duration (weeks)														
		8th	9th	10th	8th	9th	10th	8th	9th	10th	8th	9th	10th	8th	9th	10th
Body care	Mean	25.84	29.55	30.74	20.83	29.57	26.33	27.38	35.15	34.31	23.59	24.17	35.00	26.74	24.54	26.84
	SEM	3.96	3.72	4.39	2.37	4.63	4.35	4.67	3.98	10.39	2.57	3.19	9.23	3.73	2.67	4.71
Digging	Mean	18.68	19.60	16.89	12.87	14.84	25.71	22.98	25.26	22.08	28.21	25.60	17.90	19.22	25.03	16.06
	SEM	5.59	4.78	2.64	3.12	2.76	6.86	4.98	4.12	4.28	7.42	3.98	2.47	4.86	5.70	3.78
Non-social exploration	Mean	310.16	336.20	356.97	296.63	334.07	345.04	304.77	344.95	329.27	311.50	358.66	349.43	299.57	339.50	323.23
	SEM	9.83	14.88	13.14	9.01	12.67	12.00	10.29	10.79	17.14	14.00	12.52	17.52	12.18	10.72	13.55
Exploration from distance	Mean	47.96	33.07	35.04	51.07	41.47	35.12	42.10	36.60	35.73	43.43	33.71	33.34	44.10	36.49	32.97
	SEM	4.39	2.38	2.75	6.14	4.51	3.38	4.37	3.81	4.37	2.57	2.66	3.88	3.93	2.75	3.93
Social investigation	Mean	68.37	39.24	44.61	66.26	40.51	55.73	78.24	49.35	67.17	81.92	38.56	65.08	75.03	49.96	73.71
	SEM	10.79	7.89	8.36	14.17	7.51	10.14	11.05	8.59	9.82	15.28	6.96	16.52	12.59	9.06	15.54
Threat	Mean	59.52	53.06	35.19	71.27	61.25	44.25	63.71	42.15	44.08	57.13	52.17	38.09	63.05	52.45	52.10
	SEM	5.35	7.17	5.33	4.85	6.37	6.21	8.78	5.26	6.22	7.26	6.40	5.52	8.14	6.13	6.90
Attack	Mean	70.15	89.70	81.52	81.61	79.33	68.62	61.33	67.34	68.29	54.74	67.57	61.45	72.96	72.63	75.63
	SEM	9.92	12.61	13.75	12.58	8.40	10.17	11.94	10.39	15.31	10.19	12.82	9.60	14.05	8.75	11.66

Table 2. Frequency of behavioral categories displayed by TP-treated animals and controls on the 8th, 9th and 10th weeks of treatment

		Sesame oil			Treatment (mg/kg)											
		3.75			7.5			15			30					
		Frequency (weeks)														
		8th	9th	10th	8th	9th	10th	8th	9th	10th	8th	9th	10th	8th	9th	10th
Body care	Mean	16.87	25.64	28.80	16.14	22.29	24.20	17.94	26.41	23.17	16.25	20.71	26.73	18.80	21.88	21.94
	SEM	2.13	3.64	4.28	1.87	2.69	3.52	2.20	2.57	2.33	1.41	3.16	3.76	2.19	1.74	1.86
Digging	Mean	10.60	14.21	14.20	10.43	10.50	20.20	14.12	16.65	17.17	17.12	17.94	17.40	11.33	16.59	14.06
	SEM	1.85	2.97	1.64	2.15	1.88	3.53	2.58	2.24	2.95	3.64	2.41	2.20	2.10	2.72	2.88
Non-social exploration	Mean	76.33	85.79	90.87	82.43	90.43	99.60	82.06	94.23	93.78	84.25	89.65	93.67	79.00	91.00	93.75
	SEM	4.08	5.45	4.67	2.89	3.34	5.26	3.67	3.46	4.16	4.97	4.00	4.91	3.11	2.89	4.77
Exploration from a distance	Mean	46.00	42.21	40.73	49.00	48.28	44.93	44.65	45.82	41.89	50.31	44.53	40.40	45.20	44.12	40.56
	SEM	3.33	2.43	2.61	3.51	1.76	2.65	3.22	3.17	3.81	2.44	2.30	3.16	3.27	2.18	2.85
Social investigation	Mean	28.67	23.00	26.20	28.57	28.21	33.20	32.41	29.88	34.94	33.19	26.06	29.47	30.80	30.65	36.56
	SEM	3.30	3.26	3.19	4.66	4.41	4.37	2.58	3.32	3.55	3.60	3.65	3.67	3.03	2.65	3.56
Threat	Mean	38.27	40.36	33.73	46.86	46.21	35.40	39.88	36.59	37.61	35.25	39.35	34.47	39.27	39.00	40.37
	SEM	3.65	4.81	4.37	4.78	3.48	4.10	4.94	4.73	4.95	3.95	4.39	4.63	4.56	4.16	4.41
Attack	Mean	17.60	23.57	20.13	23.29	23.00	19.93	16.00	20.41	19.89	14.37	20.23	19.13	16.20	21.76	21.00
	SEM	2.27	2.26	3.13	3.14	1.79	2.66	2.59	2.53	3.41	2.06	2.97	3.08	1.89	2.77	3.07

Table 3. Latency (in seconds) of behavioral categories displayed by TP-treated animals and controls on the 8th week of treatment

Latency (8th week)		**Sesame oil**	**Treatment (mg/kg)**			
			3.75	**7.5**	**15**	**30**
Body care	Md (range)	171.48 (88.15–311.20)	91.59 (0.33–200.48)	112.38 (27.80–343.66)	94.69 (1.15–282.43)	177.57 (6.16–318.84)
Digging	Md (range)	195.26 (22.91–600)	176.56 (23.84–568.31)	210.75 (29.39–600)	124.18 (0.55–508.34)	247.44 (0.71–600)
Non-social exploration	Md (range)	0.38 (0.22–0.61)	0.33 (0.22–1.04)	0.33 (0.27–0.55)	0.35 (0.27–2.63)	0.38 (0.17–12.74)
Exploration from a distance	Md (range)	7.91 (2.25–52.62)	8.65 (2.09–21.09)	6.38 (1.37–14.78)	6.61 (2.26–50.8)	7.3 (2.09–46.91)
Social investigation	Md (range)	8.96 (5.11–23.01)	13.26 (5.10–283.03)	9.78 (1.81–288.63)	8.98 (3.24–354.27)	13.29 (0.44–30.04)
Threat	Md (range)	9.83 (3.68–265.35)	20.27 (2.8–420.62)	8.46 (3.08–567.38)	10.73 (2.64–509.88)	27.68 (4.23–156.10)
Attack	Md (range)	37.51 (14.50–365.64)	42.84 (5.54–519.70)	59.98 (7.58–600)	71.86 (6.42–600)	46.58 (2.86–600)

Table 4. Latency (in seconds) of behavioral categories displayed by TP-treated animals and controls on the 9th week of treatment

Latency (9th week)		**Sesame oil**	**Treatment (mg/kg)**			
			3.75	**7.5**	**15**	**30**
Body care	Md (range)	61.35 (8.90–201.97)	87.68 (39.98–310.39)	106.93 (6.38–275.68)	88.76 (1.64–312.25)	85.14 (8.78–251.72)
Digging	Md (range)	224.61 (58.33–429.29)	149.99 (17.57–600)	155.99 (3.3–384.86)	112.21 (12.25–435.77)	142.32 (61.73–586.34)
Non-social exploration	Md (range)	0.33 (0.27–3.57)	0.33 (0.22–0.38)	0.28 (0.22–0.72)	0.33 (0.16–0.61)	0.33 (0.22–0.44)
Exploration from a distance	Md (range)	6.26 (2.25–42.52)	3.65 (2.03–43.55)	8.3 (2.48–40.15)	8.51 (1.86–23.40)	7.25 (1.97–104.47)
Social investigation	Md (range)	64.43 (5.11–215.75)	34.215 (2.30–244.91)	53.27 (5.44–167.74)	24.94 (3.57–121.55)	50.31 (10.44–162.36)
Threat	Md (range)	11.81 (0.59–57.45)	6.87 (2.85–34.49)	9.78 (2.14–448.36)	9.94 (3.02–51.30)	11.04 (2.69–238.21)
Attack	Md (range)	15.71 (4.83–183.51)	9.33 (1.26–183.08)	12.09 (3.35–382.61)	17.52 (3.35–4.21)	12.25 (4.01–433.96)

Table 5. Latency (in seconds) of behavioral categories displayed by TP-treated animals and controls on the 10th week of treatment

Latency (10th week)		**Sesame oil**	**Treatment (mg/kg)**			
			3.75	**7.5**	**15**	**30**
Body Care	Md (range)	59.48 (14.28–152.36)	91.12 (0.44–340.64)	62.47 (22.47–199.54)	65.91 (0.88–167.14)	79.72 (17.80–302.92)
Digging	Md (range)	121.50 (45.53–440.01)	173.84 (39.48–446.43)	144.15 (45.26–600)	130.28 (22.46–305.39)	133.96 (40.43–600)
Non-social exploration	Md (range)	0.28 (0.22–0.38)	0.33 (0.11–6.10)	0.28 (0.16–0.61)	0.28 (0.17–0.38)	0.33 (0.06–0.50)
Exploration from a distance	Md (range)	5.39 (2.58–77.33)	4.12 (1.97–30.82)	4.03 (1.60–74.09)	5.11 (1.76–23.56)	4.23 (1.98–69.65)
Social investigation	Md (range)	38.39 (3.57–221.73)	66.19 (6.37–247.93)	25.27 (3.40–137.70)	12.85 (4.11–165.77)	53.28 (2.73–167.03)
Threat	Md (range)	15.87 (3.85–45.81)	5.38 (2.03–124.51)	3.79 (2.14–230.90)	9.01 (4.23–50.14)	4.97 (1.71–176.26)
Attack	Md (range)	9.28 (2.91–600)	7.42 (3.40–419.62)	9.72 (3.23–276.33)	11.04 (4.5–600)	8.85 (3.02–287.65)

Table 6. Descriptive statistics of testosterone and corticosterone levels in mice which received different doses of TP and sesame oil of grouped or isolated mice of similar age which did not receive treatment

	Testosterone (ng/l)		Corticosterone (ng/ml)	
	Mean (SD)	**Median (range)**	**Mean (SD)**	**Median (range)**
3.75 mg/kg	3.53 (4.44)	1.35 (0.2–15.3)	99.94 (71.65)	81.00 (25–287)
7.5 mg/kg	3.98 (3.62)	2.90 (0.5–13.6)	113.94 (53.89)	108.00 (37–250)
15 mg/kg	3.59 (3.25)	2.15 (0.5–12.9)	82.78 (46.85)	82.50 (14–209)
30 mg/kg	6.09 (4.20)	6.20 (1.0–16.0)	88.23 (60.32)	77.00 (21–255)
Sesame oil	3.03 (4.04)	1.00 (0.5–12.2)	87.80 (59.33)	90.00 (8–204)
Grouped	5.16 (5.11)	3.70 (0.2–18.2)	60.07 (43.62)	45.50 (17–187)
Isolated	7.77 (6.60)	7.40 (0.2–18.5)	55.40 (56.70)	33.50 (10–254)

significant. However, the latency of threat increased in the control group whereas it diminished in the treated groups. Significant decreases were found (Mann-Whitney U tests) when comparing the latency of threat in the 3.75 mg/kg ($p < .01$) and 30 mg/kg ($p < .02$) groups on the 8th week with that found on the 9th and 10th weeks. The group treated with 7.5 mg/kg showed a significant decrease when the latency shown on the 8th and 10th weeks were compared ($p < .02$).

There was a general increase in the total duration of attack (Fig. 2), except in those animals injected with the lowest dose, although it was not significant. The number of attacks significantly increased over time ($F_{2,120} = 4.95$; $p < .01$) but there was no significant 'treatment' or 'treatment × time' interaction effects. Finally, a significant reduction of the latency was found in all groups (Mann-Whitney U tests) when latency on the 8th week was compared with latency on the 9th ($p < .02$) and 10th weeks ($p < .05$).

No significant effect of TP treatment was observed in any behavioral category in each week when assessed separately (8th, 9th and 10th), except with respect to the latency of threat on the last week of treatment (Kruskal-Wallis, $p < .05$). Significant reductions (Mann-Whitney U tests) appeared in mice treated with 3.75 ($p < .02$), 7.5 ($p < .01$) and 30 mg/kg ($p < .05$). Mean and SD of total duration and frequency, and Medians, with ranges, of the behavioral categories recorded are shown in Tables 1–5.

Table 7. Mean and SEM of body, liver and testes weight (g) in mice which received TP or sesame oil

		Oil	**3.75 mg/kg**	**7.5 mg/kg**	**15 mg/kg**	**30 mg/kg**
Body	Mean	44.10	45.18	46.57	43.88	43.76
	SEM	1.41	0.95	1.14	0.80	1.20
Liver	Mean	4.78	4.83	4.74	4.72	5.05
	SEM	0.22	0.15	0.18	0.19	0.23
Testes	Mean	0.33	0.31	0.27*	0.31	0.30
	SEM	0.02	0.01	0.01	0.01	0.01

* $p < 0.05$.

Testosterone and Corticosterone Levels

Descriptive statistics of the testosterone and corticosterone levels of the different groups are presented in Table 6. Testosterone median values found in the groups treated with the three lowest doses of TP were higher than those of the control group, however, only the animals which received 30 mg/kg had values 6-fold greater than controls. With respect to corticosterone, the highest value appeared in the group treated with the 7.5 mg/kg dose. The Kruskal-Wallis test revealed a significant effect of treatment on testosterone levels ($p < .03$). Post-hoc comparisons showed that animals treated with 30 mg/kg of TP had significantly higher testosterone levels than controls ($p < .01$) and animals which received 3.75 mg/kg ($p < .02$) and 15 mg/kg ($p < .05$). No significant effect was found on corticosterone levels.

Correlations between testosterone and corticosterone levels for the total sample and for each group were calculated separately. None of them were statistically significant. Similar results were found in isolated and grouped animals.

Body and Organ Weights

Mean and SEM are shown in Table 7. Body and liver weight were unaffected by the treatment but testes weight was reduced by TP treatment ($F_{4,78} = 2.39$; $p < .05$). Post-hoc tests showed significant differences between mice treated with 7.5 mg/kg of testosterone and controls.

Relationships between Hormonal Levels and Behavioral Categories

Correlations between hormonal levels and total duration, frequency and latency of threat and attack displayed on the 10th week were calculated. There was a positive significant correlation between testosterone and total duration of threat ($r = 0.26$; $p < .05$), and corticosterone was negatively correlated with the latency of attack ($r = -0.31$; $p < .01$).

Additionally, correlations were calculated for each group separately. Since the few significant correlations found did not follow any consistent pattern, they are not included.

Individual Differences

Due to the great variability of the behavioral data, statistical analyses of the data recorded on the last week of treatment were recalculated dividing the total sample into high attacking (HA) and low attacking (LA) subjects based on whether their latency of attack on the 8th week of treatment was above or below the median for the total sample.

There were statistically significant differences in the latency of threat only among HA animals (Kruskal-Wallis, $p < .02$). Subjects treated with 3.75 ($p < .02$), 7.5 ($p < .01$) and 30 mg/kg ($p < .02$) TP showed shorter latencies than controls. However, among LA animals, there were statistically significant differences in the frequency and total duration of the non-social exploration category (Kruskal-Wallis, $p < .01$ and $< .03$, respectively). Animals which received 3.75 mg/kg TP significantly explored a higher number of times than controls ($p < .04$), and those injected with 30 mg/kg spent less time exploring the environment ($p < .03$). No differences were found in any other behavioral category, motor activity or hormonal levels in either HA or LA groups.

DISCUSSION

The 10-week treatment with TP significantly affected testis weight and serum testosterone levels but not corticosterone levels. These effects were different according to the doses: the highest dose of TP significantly increased testosterone levels while 7.5 mg/kg was the only dose which significantly decreased testis weight. This reduction seems to be due to the inhibiting power of the exogenous testosterone administration over endogenous production. The testicular reduction could be interpreted as an index of androgenic influence which has been reported in other studies (Minkin et al., 1993; Yu-Yahiro et al., 1989). Body weight was employed as a rough indicator of anabolic power, although no strong differences in function of treatment were found in agreement with other studies which also used adult non-castrated animals (Lombardo, 1993). It has been suggested that AAS only have anabolic power in adults when such factors as a hyperproteic diet and training are present (American College of Sports and Medicine, 1984; Elashoff et al., 1991). Changes in liver weight were small and non-significant although the greatest weights were found in animals treated with the highest dose which could be related to the liver toxicity found in some of the AAS users (Friedl, 1993).

The most remarkable finding in the present work is the almost total lack of effects of TP on behavior. Despite the potential promoting effect of exogenous testosterone administration on aggression, our results show no effects on attack and a slight effect on threat. This is in accordance with studies employing gonadally intact rats and different models which showed that a chronic administration of TP (15 mg/kg) facilitated threat (Lumia et al., 1994) and dominance (Bonson and Winter, 1992; Bonson et al., 1994; Lumia et al., 1994) but had no effect on attack (Lumia et al., 1994). In mice, no increases were found when using a total aggression score (Sandnabba et al., 1994). Previous studies carried out in our laboratory showed that the differential effect of AAS on threat and attack is markedly dependent on the dose and duration of treatment. Increases in latency of threat appeared in the control group, and an opposite tendency was observed in the treated groups. As can be seen, threat and attack showed a different evolution over the duration of the study. A similar profile was found by Martínez et al. (1994) using anosmic and intact opponents. Moreover, the differences between threat and attack are also supported by their patterns of relationships with hormonal levels which show that testosterone correlates with threat whereas corticosterone correlates with attack.

Additionally, the significant effects on behavior were not found in the first encounter but in the third one in the present work, and in the fourth and fifth tests in the study of Lumia et al. (1994) which suggests that agonistic experience could have an important influence on the effects of TP treatment.

There is wide agreement with regard to the influence of androgens on aggressive behavior in many species, based on the research carried out during several decades on the effects of exogenous testosterone administration in previously castrated mammals. This influence has progressively been seen to be modulated by factors such as previous experience, genetics, conditions of housing, and the type of fighting area (neutral area or home cage). In fact, the role of androgens in agonistic behavior is more complex than can be suggested by a superficial approach to the subject. Several studies have aimed at finding the relationship between endogenous testosterone levels and the frequency, latency or total duration of aggressive behavior (Albert et al., 1990; Bevan et al., 1957, 1958; Brain et al., 1971; Candland and Leshner, 1974; Dessi-Fulgheri et al., 1976; Leshner and Moyer, 1975; Leshner et al., 1973; Selmanoff et al., 1977). Based on these studies, it seems that normal

testosterone levels are necessary to display aggression, but this behavior does not increase with higher concentrations. In castrated male rats, there is an increase in aggression associated with normal testosterone levels but not with supranormal ones (Albert et al., 1990). In mice, extreme hormonal levels (above or below the mean) are related to non-aggressive behaviors (evitation) while levels close to the average are correlated to aggression (Brain et al., 1971; Candland and Leshner, 1974; Leshner and Moyer, 1975; Leshner et al., 1973). In the present work, the relation between levels of testosterone and aggression do not fit to a linear function.

The role of other hormones, mainly those of the pituitary–adrenocortical axis, has also been considered. Both gonadal and adrenal axes are modulated by factors such as social status and stress. For instance, subordinate animals show lower testosterone levels and higher corticosterone levels than dominant animals (Blanchard and Blanchard, 1988; Brain and Benton, 1983; Leshner, 1975). However, the relationship between these two hormones is complex and does not seem to be clear. They are positively correlated in some studies (Higley et al., 1992), negatively in others (Hayashi and Moberg, 1987; Norman, 1993), and not related at all in other studies (Bercovitch and Clarke, 1995; Rasmussen and Suomi, 1989; Rose et al., 1971).

The hormonal values obtained in this study were very different from those of animals of the same strain and similar age. Testosterone levels were dramatically lower than those obtained in animals isolated during 14 weeks and animals grouped during 13 weeks (Table 6). In the case of corticosterone, the results were inverse. Animals in this experiment presented higher corticosterone levels than the isolated and grouped animals. With respect to the hormonal profile of high testosterone levels and low corticosterone levels, it has been shown that exogenous testosterone depresses endogenous secretion of testosterone and corticotrophin (Alèn and Rahkila, 1988; Alèn et al., 1985). Additionally, animals in this experiment were submitted to some environmental factors considered as stressful events such as the isolation period, the number of agonistic encounters, and the handling in relation to the weekly injection, which could influence their hormonal profile. So the interaction between the above mentioned factors and drug administration could be responsible for the gonadal and adrenal activities found in our study.

The fact that the behavioral effects observed in the total sample were only found in those animals which were more aggressive, taking as a reference the latency of attack in the first encounter, points out the importance of individual differences. Thus, our results are in accordance with those of other studies, as is the case of Rejeski et al. (1988) who showed that TP treatment increased the frequency of attack but only in dominant animals. Most importantly, Van Oortmerssen, et al. (1987, 1992) have suggested that the latency of attack is more dependent on variation in responsiveness to testosterone than on baseline hormonal levels and this could be related to a difference in the number of steroid receptors in the central nervous system (Ogawa et al., 1996). In consonance with this notion, in humans, a personality profile of those AAS users who act aggressively suggests that these subjects were previously aggressive (Bond et al., 1995; Uzych, 1992).

It is worth noting that the strain and age of subjects are very important to elucidate the relationship between AAS and aggressive behavior. The OF-1 strain has been widely employed in the research about the effects of several drugs on aggression, but, to our knowledge no detailed information is available on the extent to which aggressive behavior is dependent on testosterone in this strain. It would be interesting to study this point in light of the results of Ogawa et al. (1996) who have shown that the effect of testosterone on the onset of aggression is significantly different between strains of mice, concretely

C57BL/6J and DBA/2J. Additionally, the age at which the drug is administered seems to have a very important influence. Increases in offensive behavior after an exposition to AAS during adolescence (Melloni et al., 1997) have been found in agreement with rises in aggression displayed by young although not by adult male mice which received Stanozolol (Martínez-Sanchis et al., 1996).

In summary, the relationship between TP treatment, hormonal levels and aggression is complex in non-castrated males. Firstly, the association between testosterone levels and aggressive behavior is far from being linear. Secondly, effects on threat and attack are different, attack being unaffected and threat being slightly affected by the treatment, a finding which would be interesting to study in more detail. Thirdly, high doses and other factors such as the agonistic/stress experience could be important in modulating behavioral consequences of AAS due to their action on hormonal levels. Finally, individual differences in basal aggressiveness must be taken into account to investigate the effects of AAS on aggressive behavior.

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