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Mechanisms Regulating Male Sexual Behavior in the Rat: Role of 3α- and 3β-Androstanediols[1]

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ABSTRACT

To assess whether naturally occurring 5α-androstanediols (5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol) play a role in the regulation of male sexual behavior in the rat, their capability to restore copulatory behavior in castrated animals was evaluated. Androstanediols were chronically administered either alone or in combination with 5α-dihydrotestosterone (DHT) or with estradiol-17β (E_2). Animals treated with testosterone (T), DHT, E_2, and vehicle, either alone or in different combinations, served as controls. The occurrence of mounting, intromission, and ejaculation as well as detailed parameters of copulatory behavior were recorded twice per week for 3 weeks. At the end of treatments, the weights of sex accessory organs were also recorded. When 3β,5α-androstanediol (3β-diol; 500 μg/day) was administered in combination with DHT (300 μg/day), full copulatory behavior was restored in all subjects in a manner similar to that obtained with E_2 plus DHT or T plus DHT combinations, thus indicating an estrogen-like behavioral effect of 3β-diol. Administration of 3α,5α-androstanediol (3α-diol; 500 μg/day) combined with DHT also restored sexual behavior, though to a lesser extent. When 3α-diol (500 μg/day) was simultaneously administered with E_2 (5 μg/day), the copulatory behavior of castrated animals was fully restored in a fashion similar to that observed after administration of DHT plus E_2 and T plus E_2 combinations, indicating a potent androgen-like effect of 3α-diol. The behavioral effect of 3β-diol plus E_2 was significantly less potent. When given alone, each androstanediol only partially restored copulatory behavior. Administration of 3α-diol either alone or combined with DHT or E_2 induced a significant increase in ventral prostate and seminal vesicle weight, mimicking the effects of T and DHT, whereas 3β-diol had very little effect. The results suggest that androstanediols may have a role as synergizing molecules in regulating male sexual behavior in rodents. The data also provide a possible explanation for the behavioral effects of DHT when given at supraphysiological doses.

INTRODUCTION

Even though 5α-androstane-3α,17β-diol (3α-diol) and 5α-androstane-3β,17β-diol (3β-diol), the naturally occurring reduced derivatives of 5α-dihydrotestosterone (DHT), appear to be key steroid molecules in a variety of reproductive functions, their precise role has not been completely elucidated. The enzyme-mediated formation of both androstanediols occurs in a number of target organs including the central nervous system [1–4]. In addition, the specific high-affinity binding of 3β-diol to the intracellular estrogen receptor has been well characterized in pituitary, hypothalamus, and uterus [5–7]. Furthermore, the capability of 3β-diol to induce a significant increase of estrogen-dependent progesterone receptors in hormone-sensitive tissues [8, 9], coupled with the observation that 3β-diol is able to induce precocious puberty in rodents [10, 11], clearly indicates potent, intrinsic, estrogen-like effects of this reduced DHT metabolite. In contrast, 3α-diol has very little, if any, interaction with intracellular steroid receptors [5, 8, 12]; however, it is extensively bioconverted backwards to DHT (70%) [13], which in turn binds with high affinity to the nuclear androgen receptor [14, 15]. As a consequence of its metabolic fate, the administration of 3α-diol is followed by potent androgen-like effects.

Although male sexual behavior in rodents is primarily dependent on specific testosterone (T) actions at a specific neural substrate [16–18], it is evident that a great diversity exists in the mechanisms by which steroid hormones exert their behavioral effects [16, 17, 19]. Two T metabolites, estradiol-17β (E_2) and DHT, have been studied extensively as regulators of masculine copulatory behavior [20–25], but the role of androstanediols has been neglected. The recent observations that A-ring reduction of synthetic progestins modulates the expression of their hormone-like [26–29] and behavioral [30, 31] effects has increased interest in the role of 3β- and 3α-diols in male sexual behavior. This study was intended to evaluate the ability of androstanediols to restore copulatory behavior in long-term-castrated male rats. Advantage was taken of the fact that castration results in a complete depletion of plasma and tissue content of androstanediols [32].

MATERIALS AND METHODS

Steroids

T, DHT, 3α-diol, 3β-diol, and E_2 were purchased from Sigma Chemical Co. (St. Louis, MO). Chemical purity of steroids was assessed by their melting points and chromatographic behavior. Steroids were dissolved in 10% ethanol-corn oil and administered s.c. as indicated. Injection volumes were 0.1–0.2 ml.

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TABLE 1. Parameters of sexual activity displayed by castrated male rats under the various daily steroid treatments.

	VEH + DHT[a] (n = 8)	E_2[b] + DHT[a] (n = 8)	3β-Diol[c] + DHT[a] (n = 9)	3α-Diol[c] + DHT[a] (n = 10)	T[c] + DHT[a] (n = 10)	DHT[c] + DHT[a] (n = 8)
% Ss with mount	62.5	100	100	70	100	75.0
% Ss with intromission	50.0	100*	100*	50+	100*	62.5
% Ss with ejaculation	37.5	100**	100**	50+	100**	62.5
% Tests with mount	16.1	80.4**	57.4**$^{+}$	25.0^{++}	82.8**	34^{++}
% Tests with intromission	12.5	66.1**	42.6**$^{+}$	18.3^{++}	75.0**	24^{++}
% Tests with ejaculation	7.1	58.9**	37.0**$^{+}$	18.3^{++}	65.6**	22^{++}
Interintromission interval (min)[d]	3.99 ± 2.33	1.39 ± 0.31	1.53 ± 0.21	1.35 ± 0.21	1.17 ± 0.13	1.68 ± 0.37
Hit rate[d]	0.53 ± 0.12	0.58 ± 0.03	0.62 ± 0.05	0.60 ± 0.11	0.63 ± 0.04	0.49 ± 0.04
No. intromissions preceding ejaculation[d]	11.30 ± 1.67	12.81 ± 0.93	10.05 ± 0.44	10.17 ± 0.81	13.76 ± 1.00	9.90 ± 1.28
Mount latency (min)[d]	7.22 ± 2.47	1.76 ± 0.55	5.48 ± 0.94	4.50 ± 1.34	2.09 ± 0.64	5.60 ± 1.56
Intromission latency (min)[d]	6.57 ± 2.56	2.75 ± 1.14	7.37 ± 1.39^{+}	6.30 ± 1.46	2.60 ± 0.69	4.79 ± 1.39
Ejaculation latency (min)[d]	16.79 ± 4.66	12.97 ± 2.08	14.79 ± 2.14	13.54 ± 1.97	12.94 ± 1.70	16.31 ± 3.13
Post-ejaculatory interval (min)[d]	14.18 ± 2.12	9.48 ± 0.59	15.19 ± 1.18^{++}	22.97 ± 1.99*$^{++}$	7.19 ± 0.34	11.56 ± 1.14

[a]300 μg/day.
[b]5 μg/day.
[c]500 μg/day.
[d]Mean ± SD.
*$p < 0.05$; **$p < 0.01$; as compared with the VEH + DHT-treated group.
$^{+}p < 0.05$; $^{++}p < 0.01$; as compared with the E_2 + DHT-treated group.

Animals

Subjects (Ss) were 80–90-day-old (260–290 g BW) male Wistar rats bred in our laboratory and selected on the basis of their display of the whole pattern of sexual behavior including ejaculation, in at least three screening tests. Rats were housed in individual cages with food and water available ad libitum and were maintained under a reversed light-dark cycle (0830 lights-off, 1830 lights-on). All rats were castrated under ether anesthesia at least 90 days before experiments. Evidence that no sexual activity was retained before the onset of steroid treatment was obtained in at least two behavioral tests.

Experimental Design

Rats were submitted to one of the following treatments: 1) a combination of DHT (300 μg/day) and one of the following steroids (500 μg/day): 3α-diol (n = 10), 3β-diol (n = 9), T (n = 10), DHT (n = 8), or E_2 (5 μg/day, n = 8), for 21 consecutive days; 2) E_2 (5 μg/day) combined with one of the following steroids (500 μg/day): 3α-diol (n = 9), 3β-diol (n = 10), T (n = 6), or DHT (n = 6), for 21 consecutive days; 3) one of the following steroids alone (500 μg/day): 3α-diol (n = 11), 3β-diol (n = 12), T (n = 10), or DHT (n = 11), for 21 consecutive days. Additional groups of animals that received E_2 (5 μg/day)plus vehicle (n = 8), DHT (300 μg/day) plus vehicle (n = 8), or the vehicle alone (n = 10), for 21 consecutive days, were used as negative controls; animals receiving the combined DHT plus E_2 treatments (in groups 1 and 2 above) served as positive controls.

Behavioral Assessment

Male sexual behavior was evaluated by standard techniques [16, 33–35]. Tests began on the day of the onset of treatment (Day 0) and continued thereafter twice a week until Day 21. Tests were done during the dark phase of the cycle under dim red light. Rats were placed in Plexiglas observation cages (60 × 60 × 42 cm), and after a 5-min adaptation period, each subject was presented with a receptive female. Stimulus females received 5 μg E_2 benzoate three times per week and 0.5 mg progesterone 4 h before testing. The number of mounts and intromissions as well as the mount, intromission, and ejaculation latencies, and the post-ejaculatory interval, were recorded and measured. The test was ended in one of the following circumstances: 1) 15 min after the presentation of the female to the male if no intromission occurred, 2) 30 min after the first intromission if no ejaculation had occurred, or 3) after the first intromission following ejaculation.

The rates of copulation and its efficiency were evaluated as the interintromission intervals and the "hit rates," respectively. The interintromission interval results from dividing the ejaculation latency by the number of intromissions, or by dividing 30 min by the number of intromissions when no ejaculation occurs; the hit rate results from dividing the number of intromissions by the total number of mounts plus intromissions displayed by the subject in each test; it renders an estimation of the efficiency of the consummatory mechanism [16, 33]. After completion of the last behavioral test, Ss were killed by overexposure to ether, and ventral prostate and seminal vesicles were removed and weighed to the nearest 0.1 mg.

Statistics

Proportions of sexually active animals and proportions of tests in which subjects were active were analyzed by the Fisher and X^2 tests, respectively. The number and latencies

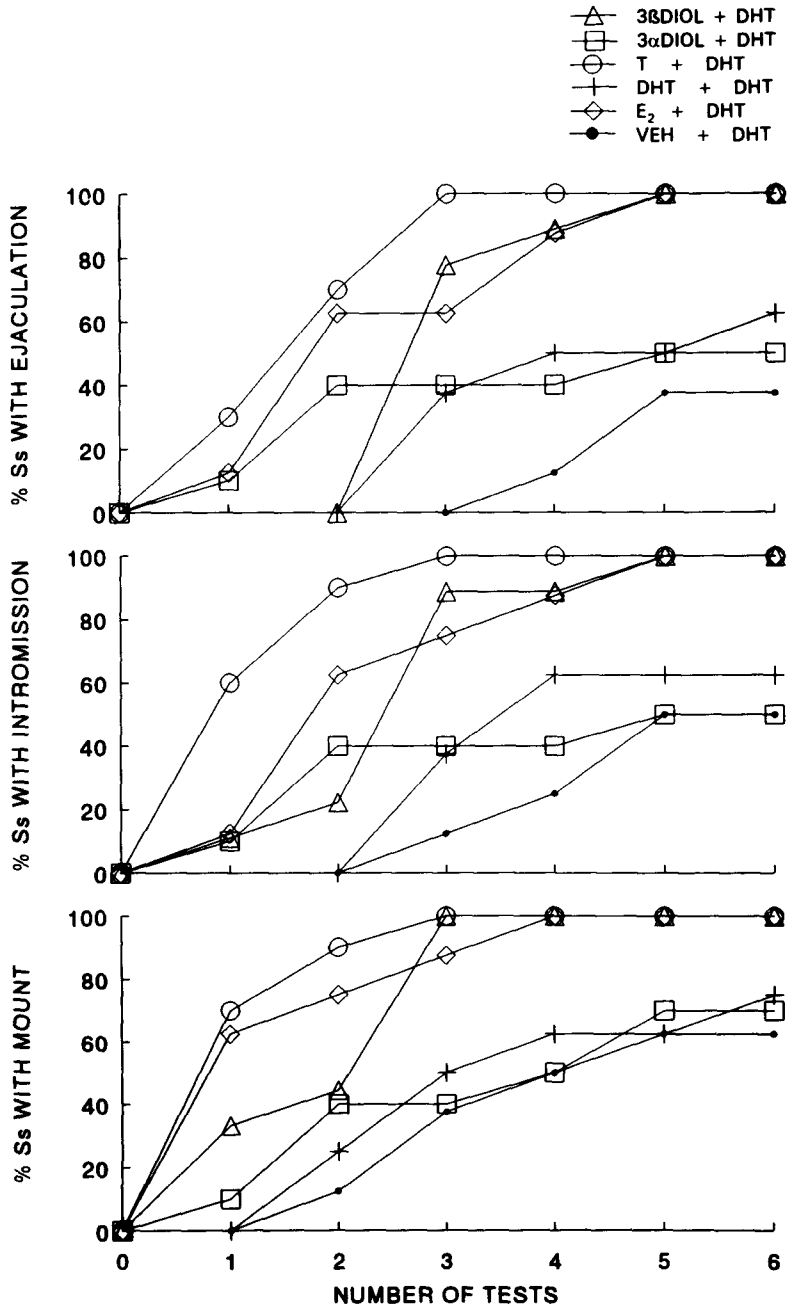

FIG. 1. Estrogen-like behavioral effects of androstanediols as assessed by their ability to synergize with DHT. Results are presented as percentages of castrated male rats showing mount, intromission, and ejaculation during 21 days of treatment with T, DHT, 3α-diol, or 3β-diol (500 μg/day) in combination with DHT (300 μg/day). Castrated rats treated with either E_2 (5 μg/day) or vehicle (VEH) + DHT (300 μg/day) served as controls. 3β-diol restored full copulatory behavior when given with DHT.

of behavioral responses were analyzed by the Mann-Whitney "U" test; organ weights were analyzed by Student's *t*-test. Group differences were considered significant when $p < 0.05$ (two-tailed test) [36].

RESULTS

The behavioral effects of androstanediols (3α-diol; 3β-diol) administered in combination with DHT to adult male castrated rats are shown in Figure 1. The combined administration of 3β-diol + DHT fully restored the copulatory activity in all Ss, in a manner similar to that observed after administration of E_2 + DHT. All subjects receiving 3β-diol + DHT displayed a complete pattern of sexual behavior (mounting, intromission, and ejaculation) in a proportion of tests significantly higher ($p < 0.01$) than those exhibited by animals treated with DHT + vehicle, as indicated in Ta-

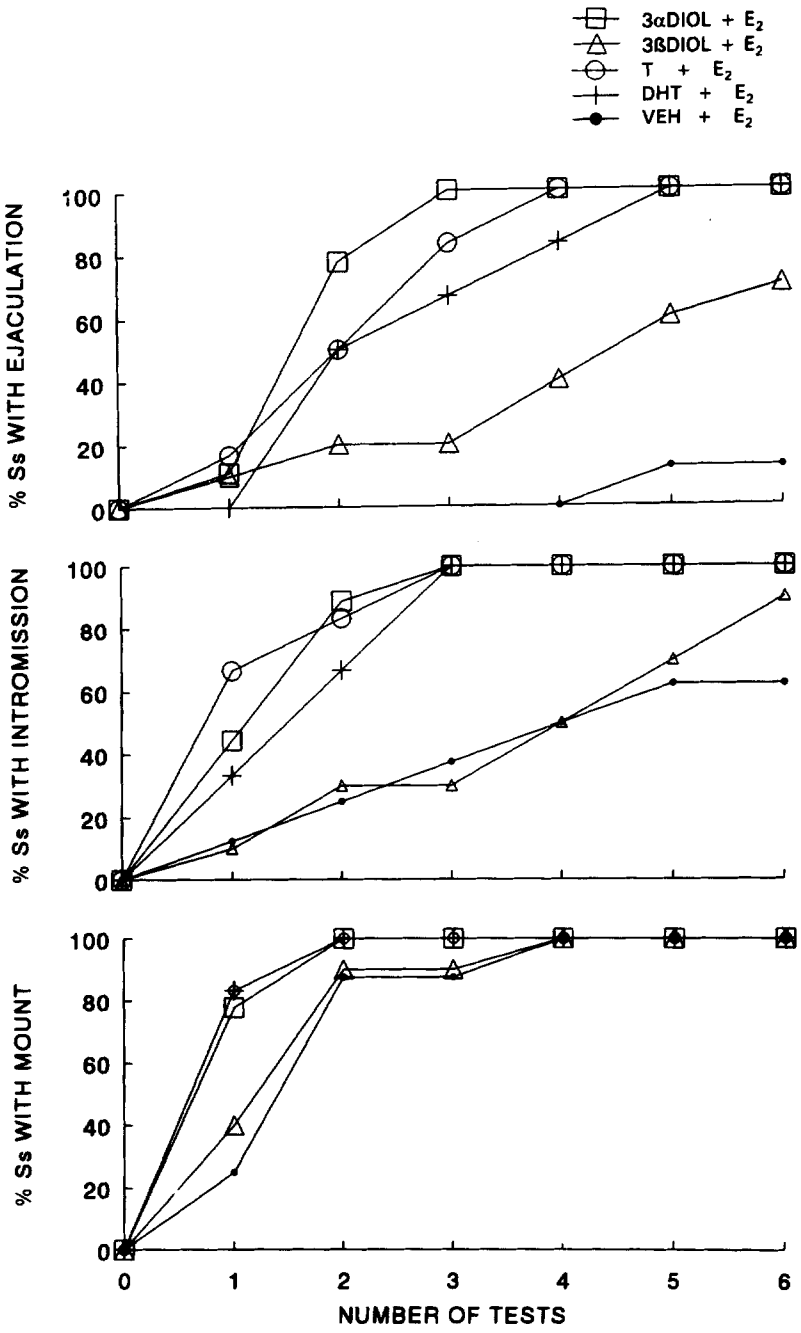

FIG. 2. Androgen-like behavioral effects of androstanediols as assessed by their ability to synergize with E_2. Results are presented as percentages of castrated male rats showing mount, intromission, and ejaculation during 21 days of treatment with T, DHT, 3α-diol, or 3β-diol (500 μg/day) in combination with E_2 (5 μg/day). Castrated rats treated with vehicle (VEH) + E_2 (5 μg/day) served as controls. 3α-diol restored full copulatory behavior when given with E_2.

ble 1; however, the incidence of sexual activity induced by 3β-diol + DHT was lower than that induced by E_2 + DHT. Analysis of detailed parameters of sexual activity demonstrated that animals treated with 3β-diol + DHT scored similar values of interintromission intervals, hit rates, number of intromissions preceding ejaculation, and ejaculation latencies compared to those observed in the positive control animals treated with E_2 + DHT (Table 1); however, the mount and intromission latencies as well as the post-ejaculatory intervals were longer in the experimental group.

In sharp contrast, administration of 3α-diol combined with DHT to castrated male rats resulted in a weak sexual behavioral response. Indeed, only 50% of animals receiving this treatment presented intromissions and ejaculations (Fig. 1; Table 1). Moreover, these behavioral responses were displayed in less than 20% of the tests. In the few circum-

TABLE 2. Parameters of sexual activity displayed by castrated male rats under the various daily steroid treatments.

	VEH + E_2[a] (n = 8)	T[b] + E_2[a] (n = 6)	DHT[b] + E_2[a] (n = 6)	3α-Diol[b] + E_2[a] (n = 9)	3β-Diol[b] + E_2[a] (n = 10)
% Ss with mount	100	100	100	100	100
% Ss with intromission	62.5	100	100	100	90
% Ss with ejaculation	12.5	100**	100**	100**	70*
% Tests with mount	78.6	94.4	94.4	92.6	75.0^{+}
% Tests with intromission	25.0	88.9**	77.8**	81.5	38.3^{++}
% Tests with ejaculation	1.8	72.2**	58.3**	68.5**	28.3**$^{++}$
Interintromission interval (min)[c]	7.53 ± 2.26	1.11 ± 0.24**	1.98 ± 0.53*	1.68 ± 0.19*	3.24 ± 1.54*
Hit rate[c]	0.26 ± 0.06	0.59 ± 0.04**	0.55 ± 0.07**	0.55 ± 0.03**	0.42 ± 0.07
No. intromissions preceding ejaculation[c]	20.0	10.92 ± 1.47	12.02 ± 0.87	10.91 ± 0.80	11.51 ± 0.93
Mount latency (min)[c]	3.28 ± 1.0	0.98 ± 0.27*$^{+}$	2.37 ± 0.46	1.01 ± 0.15**$^{+}$	2.94 ± 0.99
Intromission latency (min)[c]	3.95 ± 0.95	1.89 ± 0.67	3.32 ± 0.68	2.11 ± 0.42	4.17 ± 1.73
Ejaculation latency (min)[c]	29.0	9.80 ± 2.34	11.95 ± 2.3	13.27 ± 1.81	14.17 ± 2.92
Post-ejaculatory interval (min)[c]	8.67	6.92 ± 0.44^{+}	9.70 ± 1.14	10.46 ± 0.99	9.15 ± 1.58

[a]5 μg/day.
[b]500 μg/day.
[c]Mean ± SD.
*$p < 0.05$; **$p < 0.01$; as compared with the VEH + E_2-treated group.
$^{+}p < 0.05$; $^{++}p < 0.01$; as compared with the DHT + E_2-treated group.

stances in which copulatory behavior was accomplished, some of the detailed characteristics of sexual activity were similar to those found in the 3β-diol + DHT group, but with longer post-ejaculatory intervals.

When T was simultaneously administered with DHT to castrated rats, a full restoration of sexual activity occurred in all animals in a manner identical to that observed in the 3β-diol + DHT and E_2 + DHT groups, as depicted in Figure 1. Analysis of the incidence of sexual activity and behavioral parameters showed that animals treated with T + DHT displayed sexual behavior with identical characteristics to those of animals treated with E_2 + DHT (Table 1). A better copulatory performance, in terms of intromission latencies and post-ejaculatory intervals, was achieved in the T + DHT group than in the 3β-diol + DHT group.

The behavioral potency of androstanediols when given simultaneously with E_2 to castrated rats is shown in Figure 2. Administration of 3α-diol + E_2, but not 3β-diol + E_2, induced full restoration of copulatory activity in all animals in a fashion similar to that observed in animals treated with DHT + E_2, or T + E_2. Furthermore, the combination of 3α-diol with E_2 induced an earlier restoration of ejaculation than did T + E_2 or DHT + E_2 (Fig. 2). Detailed analysis of behavioral parameters and the incidence of sexual activity also indicated a copulatory performance of subjects receiving 3α-diol + E_2 identical to that of those receiving DHT + E_2, or T + E_2. In contrast, 3β-diol combined with E_2 induced a limited restoration of sexual behavior (Fig. 2, Table 2). Indeed, ejaculatory responses with this treatment were achieved in less than 30% of tests.

Administration of either 3α-diol or 3β-diol alone to castrated rats elicited a limited display of copulatory behavior compared to that of animals treated with T alone (Fig. 3). Animals treated with 3α-diol exhibited earlier restoration of ejaculation and more efficient copulatory behavior than those treated with its 3β-isomer (Table 3). Administration of 3α-diol alone induced a better copulatory performance, as assessed by hit rate values, than DHT or 3β-diol given at similar doses (Table 3). When DHT was administered alone, at different dose levels (control groups), it elicited a limited restoration of sexual behavior; however, a dose-response tendency was observed (Tables 1 and 3).

The effects of androstanediols on accessory sex gland weights are shown in Figure 4. When given alone, 3α-diol induced a significant increase on ventral prostate and seminal vesicle weights compared with T ($p < 0.01$) and with 3β-diol ($p < 0.001$). The effect of 3α-diol on accessory sex glands was similar to or even more potent than that found with DHT. The simultaneous administration of 3α-diol, but not 3β-diol, with DHT induced an additive effect in terms of ventral prostate weight. Administration of 3α-diol + E_2 resulted in a significant increase ($p < 0.001$) in accessory sex gland weight compared to that of animals treated with 3β-diol + E_2, or E_2 alone (Fig. 4). The effects observed with the 3α-diol + E_2 combination were similar to those observed with T + E_2 and DHT + E_2.

DISCUSSION

The results reported here provide evidence that two naturally occurring androstanediols exhibit hormone-like behavioral effects in the long-term-castrated male rats. Indeed, the simultaneous administration of 3β-diol with DHT fully restored copulatory behavior in castrated animals, in a manner comparable to that observed in animals treated with E_2 + DHT. These results indicate that 3β-diol mimics the behavioral effects of E_2 and are consistent with the reports of a number of laboratories showing high-affinity binding of 3β-diol to the estrogen receptor [5–7, 37, 38].

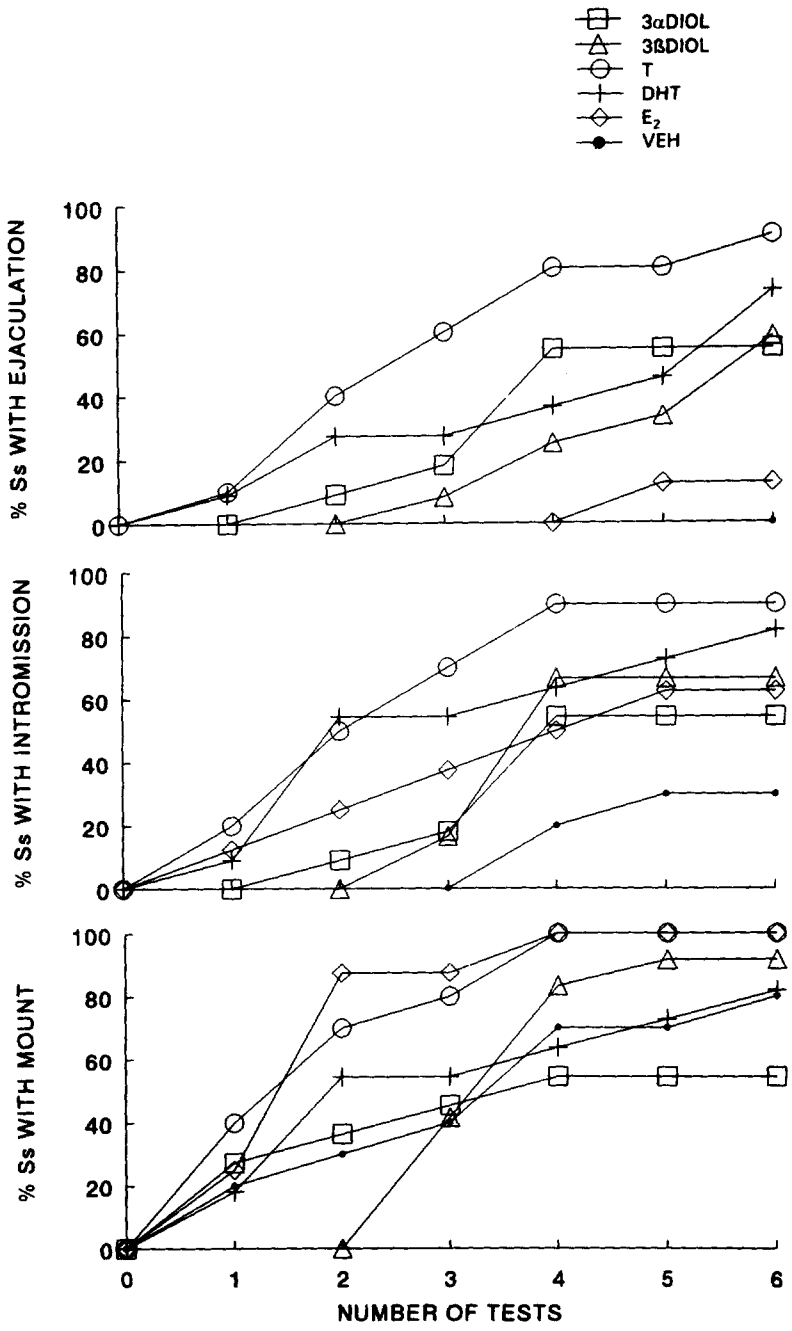

FIG. 3. Intrinsic behavioral effects of androstanediols. Results are presented as percentages of castrated male rats showing mount, intromission, and ejaculation during 21 days of treatment with 3α-diol, 3β-diol, T, DHT (500 μg/day), or E_2 (5 μg/day). Castrated rats treated with vehicle (VEH) served as controls. Neither 3α-diol nor 3β-diol restored full copulatory behavior in subjects.

The observation that 3β-diol + DHT elicited sexual behavior in all treated Ss, with values of interintromission intervals, intromissions preceding ejaculation, and ejaculation latencies similar to those presented by animals treated with E_2 + DHT, confirms and extends the previous report by Baum and Vreeburg [39], who, using higher doses of 3β-diol, demonstrated a synergistic action with DHT propionate. Overall, the data demonstrate that 3β-diol induces an estrogenic effect upon the neural substrate responsible for male sexual behavior activation, in addition to its well-known estrogenic action at peripheral tissues [8, 9]. In contrast, administration of 3α-diol with DHT failed to restore efficient copulatory behavior in castrated rats. This observation correlates with the lack of interaction of 3α-diol with the estrogen receptor [5, 38]. Interestingly, the behavioral synergizing effect of 3β-diol with DHT resembles that of the

TABLE 3. Parameters of sexual activity displayed by castrated male rats under the various daily steroid treatments (500 μg/day for 21 days).

	VEH (n = 10)	T (n = 10)	DHT (n = 11)	3α-Diol (n = 11)	3β-Diol (n = 12)	E_2 (5 μg/day) (n = 8)
% Ss with mount	80	100	81.8	54.5^{+}	91.66	100
% Ss with intromission	30	90*	81.8*	54.5	66.66	62.5
% Ss with ejaculation	—	90**	72.7**	54.5**	58.33**	12.5
% Tests with mount	22.86	72.8**	37.66^{++}	28.57^{++}	38.09^{++}	78.6
% Tests with intromission	4.28	61.4**	33.76**$^{++}$	20.78**$^{++}$	25.00**$^{++}$	25.0
% Tests with ejaculation	—	52.8**	22.07**$^{++}$	20.78**$^{++}$	14.28**$^{++}$	1.8
Interintromission interval (min)[a]	12.50 ± 3.5	1.33 ± 0.84*	2.49 ± 0.41^{+}	1.22 ± 0.15*	2.01 ± 0.28*	7.53 ± 2.26
Hit rate[a]	0.12 ± 0.05	0.60 ± 0.16*	0.56 ± 0.05*	0.70 ± 0.05*	0.47 ± 0.05*	0.26 ± 0.06
No. intromissions preceding ejaculation[a]	—	13.60 ± 2.20	12.02 ± 1.01	12.60 ± 0.41	11.17 ± 1.44	20.0
Mount latency (min)[a]	8.26 ± 1.77	2.79 ± 0.75*	4.06 ± 0.98	2.94 ± 0.49	3.87 ± 0.93	3.28 ± 1.0
Intromission latency (min)[a]	8.13 ± 3.13	2.87 ± 0.75	5.18 ± 1.22	4.73 ± 1.02	6.23 ± 1.02^{+}	3.95 ± 0.95
Ejaculation latency (min)[a]	—	14.15 ± 1.75	18.85 ± 1.35	15.35 ± 1.86	15.32 ± 3.09	29.0
Post-ejaculatory interval (min)[a]	—	7.66 ± 0.64	15.60 ± 1.18^{++}	18.55 ± 2.32^{++}	11.16 ± 1.17^{+}	8.67

[a]Mean ± SD.
*$p < 0.05$; **$p < 0.01$; as compared with the VEH-treated group.
$^{+}p < 0.05$; $^{++}p < 0.01$; as compared with the T-treated group.

3β,5α-tetrahydro derivative of norethisterone (NET) reported by our laboratory [30]. The synthetic 3β,5α-NET molecule shares with 3β-diol structural similarities, binding affinities to the estrogen receptor, and behavioral estrogenic potencies [26, 27, 30].

To determine whether androstanediols may exhibit androgenic behavioral effects, they were simultaneously administered with E_2 to castrated male rats. The results demonstrated that 3α-diol induced full restoration of masculine behavioral activity in all treated animals, with a better copulatory performance than that displayed by animals treated with DHT + E_2, or T + E_2. These data demonstrate a potent androgen-like behavioral effect of 3α-diol, in addition to its well-known androgenic potency in peripheral organs [40–42]. Since 3α-diol is unable to bind to the androgen receptor in hormone-sensitive and hormone-dependent tissues [5, 8, 12] its mechanisms of androgenic action seem to be mediated by its extensive bioconversion to DHT [13]. The finding that 3β-diol, when given simultaneously with E_2, induced only a limited restoration of copulatory behavior in castrated rats, is in line with the observation that 3β-diol is not as efficiently back-converted to DHT as the 3α-isomer [13].

The potent hormone-like behavioral effects of androstanediols found in these experiments prompted us to assess their intrinsic behavioral potency by administering them alone to castrated male rats. The results demonstrated that neither 3α-diol nor 3β-diol, at the dose level used, were able to restore full copulatory behavior in treated subjects. These data are consistent with the previous observation by Parrott [43] that androstanediols fail to maintain the post-ejaculatory interval values after castration in adult male rats. Similar results have been reported in other species [44].

Further evidence of the androgen-like potency of 3α-diol stemmed from the effects observed in accessory sex gland weight following its administration, either alone or in combination with DHT or with E_2. Thus, 3α-diol alone induced an increase in ventral prostate and seminal vesicle weight with a higher potency than T and DHT. In contrast, 3β-diol displayed very little, if any, androgenic activity in accessory sex glands.

The data indicate that the hormone-like effects of androstanediols at the central nervous system are similar to those observed in peripheral target organs.

The clearcut hormone-like behavioral effects of androstanediols demonstrated in this study indicate that they may play an important role in the regulation of copulatory behavior in the rat. Androstanediols act as synergizing molecules, mimicking the effects of T and/or estradiol, which under physiological circumstances in the adult animal appear to be the key agents responsible for activating the neurons involved in male sexual behavior [18–23]. In addition, and perhaps more important, the physiological relevance of androstanediols in the regulation of male copulatory behavior resides in their relative abundance at the onset of puberty. Corpechot et al. [45] demonstrated that plasma androstanediols are present in newborn rats and then in the first weeks of life became undetectable; however, a striking rise in the circulating concentrations of 3α- and 3β-diols occurs in wks 6–8, coinciding with the onset of puberty. It must be stressed that at this particular life stage, the plasma concentrations of A-ring reduced androgens, including DHT and androstanediols, are significantly higher than that of T [45]. Moreover, Eckstein et al. [11] have demonstrated that administration of 3β-androstanediol induced a true precocious puberty in immature albino rats, including vaginal opening followed by ovulatory estrous cycles. These observations, coupled with the finding that androstanediols possess potent synergizing behavioral effects, strongly suggest that these naturally occurring 5α-reduced C-19 steroids may play a key role in the onset of neural activation of copulatory behavior. Further support to this proposal is fur-

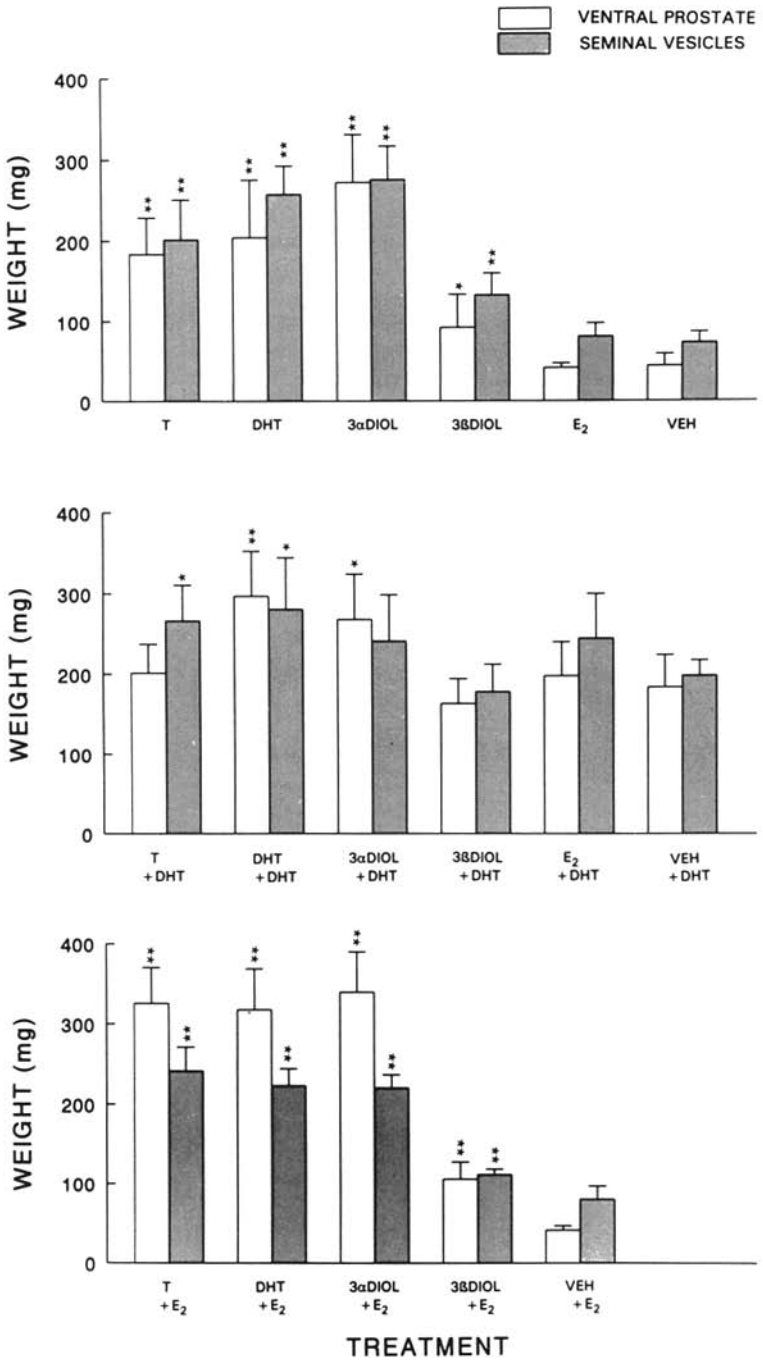

FIG. 4. Peripheral androgenic effects of androstanediols. Effects of 21 days of daily treatment with 3α-diol, 3β-diol, T, DHT (500 μg), or E_2 (5 μg), alone or in combination with either DHT (300 μg) or E_2 (5 μg), on accessory sex organ weight. Castrated animals treated with DHT (300 μg) + E_2 (5 μg), DHT (300 μg) + vehicle (VEH), E_2 (5 μg) + VEH, or VEH alone served as controls. * $p < 0.01$; ** $p < 0.001$ compared with the negative control group.

nished by the studies of Sodersten et al. [46] and Sachs and Meisel [47], who have demonstrated that sexual behavior activity in the pubertal rat begins before maximal blood levels of T are reached.

Interestingly, a biosynthetic pathway from progesterone to androstanediols, not involving T, has been described in immature rat testes by Yamada and Matsumoto [48]. Androstanediols of gonadal and extragonadal origin [49–52] may also have a role in the regulation of male copulatory behavior in the adult animal. The behavioral synergizing effects of androstanediols may explain the observation that DHT alone, given at high doses, maintains/restores copulatory behavior after castration in adulthood as reported in several mammalian species [19, 53–55].

In conclusion, the results demonstrate that androstanediols are involved in alternate mechanisms regulating masculine sexual behavior. The data also support the concept that there are diverse mechanisms in the hormonal regulation of copulatory behavior in rodents.

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