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# Induction of Male Sexual Behavior by Norethisterone: Role of Its A-Ring Reduced Metabolites

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MORALÍ, G., A. E. LEMUS, M. V. OROPEZA, G. A. GARCÍA AND G. PÉREZ-PALACIOS. *Induction of male sexual behavior by norethisterone: Role of its A-ring reduced metabolites.* PHARMACOL BIOCHEM BEHAV **37**(3) 477–484, 1990.—The estrogenic and androgenic potencies of norethisterone (NET), a synthetic nonaromatizable progestin, and three of its reduced metabolites (5α-NET; 3α, 5α-NET; 3β,5α-NET) were assessed by their ability to restore male sexual behavior in castrated male rats following their chronic administration in combination with either 5α-dihydrotestosterone (DHT) or estradiol ($E_2$), or when given alone. Full restoration of mating was achieved when 3β,5α-NET was administered with DHT, indicating an estrogenic effect of this compound. Lower estrogenic effects were noticed with 3α,5α-NET and 5α-NET, while NET had very little estrogenic potency. The only effective compound to restore ejaculation, when administered with $E_2$, was NET, indicating its androgen-like intrinsic potency. When administered alone, NET exerted the most potent effect on male behavior, followed by 5α-NET, while the tetrahydro derivatives were ineffective. The observation that NET alone restored male sexual activity at a level identical to that induced by testosterone demonstrated an androgenic-estrogenic activity of this progestin exerted through its intrinsic androgenic effect, and the estrogenic effect of its tetrahydro derivatives. Overall results indicated that the metabolism of NET modulates its mode of action at the brain, and support the concept that both estrogenic and androgenic effects are required for mating activation.

Norethisterone Sexual behavior Progestins Progestin metabolism 19-Nor steroids

NORETHISTERONE (NET), a widely used synthetic contraceptive progestin, has been shown to exert estrogenic and androgenic effects in addition to its progestational activity when administered to a number of mammalian species (7, 8, 12). The mechanisms of action of this steroid have been recently assessed in a variety of biological mammalian systems (9–12, 20, 21, 28). The results obtained have demonstrated that the enzyme-mediated conversion of NET to its A-ring reduced metabolites plays an essential role in the expression of its hormone-like activity (13). Although NET exerts its main effects via the progesterone receptor, it is effectively biotransformed at the target organs to 5α-dihydro NET (5α-NET), and subsequently to the 3α,5α- and the 3β,5α-NET tetrahydro derivatives (3α,5α-NET and 3β,5α-NET respectively) (22). The metabolic conversion products of NET interact with putative steroid binding sites other than those of the progesterone receptor; thus, 5α-NET specifically binds with high affinity to the androgen receptor, while the two tetrahydro NET metabolites specifically interact with estradiol binding sites (9). Furthermore, the 3β,5α-NET and its 3α-epimeric alcohol are able to initiate estrogen-dependent cellular responses (10,28). These metabolic events are relevant to understand the estrogen-like mode of action of NET, particularly since this synthetic progestin neither interacts with the intracellular estrogen receptor (9,27) nor undergoes aromatization in vivo in the nonpregnant condition (5,6). These data strongly suggest that the estrogenic activity of NET is mediated by their nonphenolic A-ring reduced metabolites.

To assess whether these peripheral hormonal effects of NET and its neutral, nonaromatizable derivatives are also exerted at the central nervous system (CNS), we decided to investigate their effectiveness in the activation of the neural substrate responsible for the expression of masculine sexual behavior in the castrated male rat. Advantage was taken from the fact that spontaneous male sexual behavior in rodents is under strict steroid hormone regulation. Even though the precise role of androgens and/or estrogens has remained as a controversial issue (2, 3, 17–19, 23, 29), recent evidences have indicated that androgens [testosterone or 5α-dihydrotestosterone (DHT)], acting via androgen receptors, do appear to play a pivotal role in restoring the complete pattern

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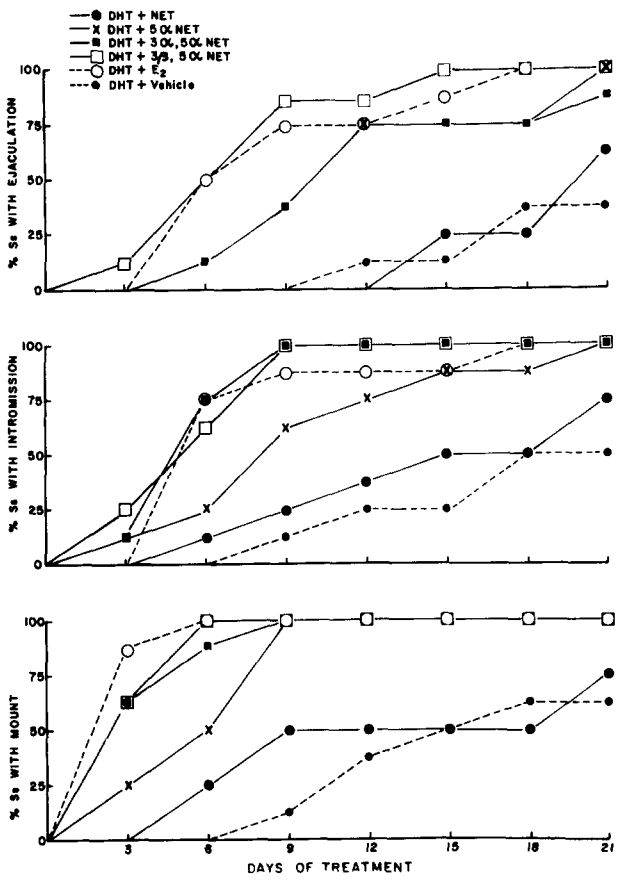


FIG. 1. Estrogen-like behavioral effects of norethisterone (NET) and its metabolites in long-term castrated male rats. Cumulative percentage of animals showing mounting, intromission and ejaculation behavior during 21 days of treatment with NET or its A-ring reduced derivatives (300 μg) in combination with dihydrotestosterone (DHT, 300 μg). Castrated animals treated with estradiol ($E_2$, 5 μg) plus DHT (300 μg) or with vehicle (10% ethanol-corn oil) plus DHT (300 μg) served as controls.

of sexual behavior in castrated male rats (16). However, a possible complementary role of estradiol (or other estrogenic compounds) acting via estradiol receptors may be also essential for mating activation (1, 14–17, 29). The behavioral effects of NET and its derivatives were assessed by giving them alone, as well as by their capability to synergize with either estradiol ($E_2$) or 5α-dihydrotestosterone.

## METHOD

### *Steroids*

Testosterone, DHT, and $E_2$ were purchased from Sigma Chemical Co. (St. Louis, MO). Authentic NET (17α-ethynyl, 17β-hydroxy-4-estren-3-one) was kindly provided by Schering Mexicana, S.A. (Mexico City). The 5α-reduced derivative of NET was synthetized by lithium-ammonia reduction of NET, as described by Bowers *et al.* (4). The 3α,5α- and 3β,5α-NET derivatives were prepared from 5α-NET by sodium borohydride reduction (4). The epimeric alcohols were separated by flash chromatography (26) using the system ethyl acetate:hexane (3:7, v/v). Chemical purity of NET and its derivatives was assessed as previously described (28) by their melting points, high performance liquid chromatographic behavior, and H-nuclear magnetic resonance spectrometric analysis.

### *Animals*

Subjects (Ss) were adult male Wistar rats bred in our laboratory and selected on the basis of a high level of sexual behavior. Animals were housed in individual cages with food and water available ad lib and were maintained under a reversed light-dark cycle (08:30 off, 18:30 on). Subjects were castrated under ether anesthesia at least 60 days prior to experiments. Evidence that no sexual activity was retained before steroid treatment was obtained in at least two behavioral tests.

### *Experimental*

All steroids including NET and its derivatives were dissolved in 10% ethanol-corn oil. Injection volumes were 0.1–0.2 ml. Subjects were assigned to one of the following experimental groups: Group 1 received SC the combination of DHT (300 μg/day) and one of the following steroids (300 μg/day): NET (n = 8), 5α-NET (n = 8), 3α,5α-NET (n = 8), or 3β,5α-NET (n = 8) for 21 consecutive days. Group 2 received SC the combination of $E_2$ (5 μg/day) and one of the following steroids (300 μg/day): NET (n = 9), 5α-NET (n = 10), 3α,5α-NET (n = 9), or 3β,5α-NET (n = 10) for 21 consecutive days. An additional group of animals (n = 10) received, for 21 days, the combination of $E_2$ and NET at the daily dose of 5 μg and 500 μg respectively. Group 3 received SC NET (n = 10), 5α-NET (n = 10), or 3β,5α-NET (n = 10) at a daily dose of 500 μg for 21 days. A group of animals (n = 8) which received the combination of DHT (300 μg/day) plus $E_2$ (5 μg/day) for 21 days was used as the experimental control for groups 1 and 2. A group of subjects (n = 10) treated with testosterone (500 μg/day) for 21 days served as the experimental control for group 3. Animals that received $E_2$ only (5 μg/day, n = 8), DHT only (300 μg/day, n = 8), and the vehicle (10% ethanol-corn oil, n = 10) for 21 days, were used as negative controls. Doses of NET and its A-ring reduced derivatives used throughout this study were determined from the results of a pilot study administering different dose levels of these compounds (150, 300, and 500 μg/day for 21 days), as well as from previous studies (10,28).

### *Behavioral Assessment*

Male sexual behavior was evaluated by standardized techniques (2, 18, 24). Tests began on the day of the onset of treatment (day zero) and continued thereafter every 72 hr until day 21. Tests were done during the dark phase of the cycle under dim red light. Subjects 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 four hr before testing. The number of mounts and intromissions as well as the mount, intromission, and ejaculation latencies, and the postejaculatory interval were recorded and measured. The test was ended in one of the following circumstances: (a) 15 min after the presentation of the female to the male if no intromission had occurred at that time, (b) 30 min after the first intromission if no ejaculation had occurred, or (c) after the first intromission following ejaculation.

The rate of copulation and its efficiency were evaluated as the interintromission interval and the hit rate respectively. The interintromission interval results from dividing the ejaculation latency by the number of intromissions including that occurring at ejaculation, or by dividing 30 min by the number of intromissions

TABLE 1

PARAMETERS OF SEXUAL ACTIVITY DISPLAYED BY CASTRATED MALE RATS UNDER THE VARIOUS DAILY STEROID TREATMENTS

| | Vehicle+DHT[a] (n=8) | $E_2$[b]+DHT[a] (n=8) | NET[a]+DHT[a] (n=8) | 5α-NET[a]+DHT[a] (n=8) | 3α,5α-NET[a]+DHT[a] (n=8) | 3β,5α-NET[a]+DHT[a] (n=8) |
|---|---|---|---|---|---|---|
| % Ss With Mount | 63 | 100 | 75 | 100 | 100 | 100 |
| % Ss With Intromission | 50 | 100* | 75 | 100* | 100* | 100* |
| % Ss With Ejaculation | 38 | 100* | 63 | 100* | 88 | 100* |
| % Tests With Mount | 18 | 96‡ | 23 | 64‡ | 73‡ | 93‡ |
| % Tests With Intromission | 14 | 77‡ | 18 | 52‡ | 57‡ | 80‡ |
| % Tests With Ejaculation | 7 | 63‡ | 11 | 41‡ | 43‡ | 55‡ |
| Interintromission Interval (sec)[c] | 116 ± 73 | 65 ± 22 | 92 ± 23 | 76 ± 34 | 113 ± 99 | 72 ± 36 |
| Hit Rate[c] | 0.61 ± 0.24 | 0.70 ± 0.15 | 0.58 ± 0.19 | 0.60 ± 0.21 | 0.60 ± 0.21 | 0.70 ± 0.18 |
| No. Intromissions Preceding Ejaculation[c] | 10.70 ± 2.50 | 12.80 ± 2.20 | 10.60 ± 3.80 | 11.30 ± 2.80 | 11.70 ± 4.20 | 11.50 ± 2.40 |
| Mount Latency (sec)[c] | 419 ± 335 | 14 ± 12 | 37 ± 21* | 36 ± 51† | 30 ± 45* | 21 ± 19† |
| Intromission Latency (sec)[c] | 350 ± 294 | 127 ± 157 | 109 ± 107 | 79 ± 80 | 124 ± 127 | 28 ± 22† |
| Ejaculation Latency (sec)[c] | 990 ± 465 | 839 ± 304 | 902 ± 181 | 944 ± 294 | 885 ± 358 | 885 ± 455 |
| Post Ejaculatory Interval (sec)[c] | 826 ± 209 | 468 ± 83 | 685 ± 248 | 606 ± 149 | 685 ± 108 | 574 ± 109 |

[a]300 μg.
[b]5 μg.
[c]Mean ± SD.
*$p<0.05$; †$p<0.01$; ‡$p<0.001$ as compared with the vehicle+DHT-treated control group.

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 a given test and it renders an estimation of the efficiency of the consummatory mechanism (14,24). 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 Ss were active, were analyzed by the Fisher and the $\chi^2$ test, while the number and latencies of behavioral responses and organ weights were analyzed by the Mann-Whitney U-test and the Student's *t*-test respectively. Group differences were considered significant when $p<0.05$ was reached (two-tail test) (25).

## RESULTS

The effects of NET and its A-ring reduced derivatives on the stimulation of male sexual behavior, when administered simultaneously with DHT to adult castrated male rats, are shown in Fig. 1. As depicted in this figure, the combined administration of the 3β,5α-tetrahydro derivative of NET with DHT fully restored the pattern of sexual behavior of all subjects in a fashion similar to that observed following the administration of $E_2$ plus DHT. The 3α,5α derivative of NET and 5α-NET also synergized with DHT in eliciting full copulatory behavior, though the ejaculatory response exhibited a slow temporal restoration (Fig. 1). Unmodified NET combined with DHT did not elicit significant male sexual behavior restoration in the castrated animals.

The detailed analysis of behavioral parameters (Table 1) disclosed that the combination of 3β,5α-NET with DHT induced mounting, intromission, and ejaculation activities in a proportion of tests similar to that of the $E_2$ plus DHT control treatment. Furthermore, animals treated with 3β,5α-NET plus DHT presented mount latencies and postejaculatory intervals almost identical to those found in the $E_2$ plus DHT control treatment. Animals treated with either 3α,5α-NET or 5α-NET combined with DHT also exhibited copulatory activity parameters comparable to those of the $E_2$-DHT-treated group, although in a lower proportion of tests (Table 1). The combined administration of NET with DHT did not stimulate significantly the incidence of male copulatory behavior as reflected in the low percentage of tests with activity. It must be stressed, however, that in those tests in which sexual activity was shown, the mount latency was significantly lower than that observed in the oil-DHT-treated group (Table 1). The NET-DHT-treated group exhibited a diminution ($p<0.05$) on the ventral prostate weight as compared with both $E_2$-DHT and oil-DHT control groups (Fig. 2). In addition, the 5α-NET-DHT-treated group presented an increase ($p<0.05$) on the seminal vesicles weight as compared with the oil-DHT control group (Fig. 2). When NET and its neutral reduced metabolites were administered simultaneously with $E_2$, only unmodified NET was able to elicit a full male copulatory pattern (Fig. 3); however, its effectiveness was below the level observed in the DHT plus $E_2$ control group. Interestingly, administration of 5α-NET with $E_2$ induced intromission behavior in most Ss but ejaculatory activity was not accomplished. On the contrary, the 3α,5α- and 3β,5α-tetrahydro derivatives of NET did not synergize at all with $E_2$ on restoring male copulatory behavior (Fig. 3).

As it can be seen in Table 2, only NET-$E_2$-treated Ss exhibited a significant increase in the proportion of tests with intromission and ejaculation activities as compared with those observed in the oil-$E_2$ control group; however, these values were lower than those of the DHT-$E_2$-treated animals. Analysis of the hit rate revealed that NET, when given with $E_2$, exhibited a moderate androgenic potency, while 5α-NET, 3α,5α-NET, and 3β,5α-NET were devoid of such effect (Table 2). Furthermore, when NET was administered at a higher dose (500 μg/day) with $E_2$ (5 μg/day), the number of intromissions preceding ejaculation (11.7 ± 0.6;

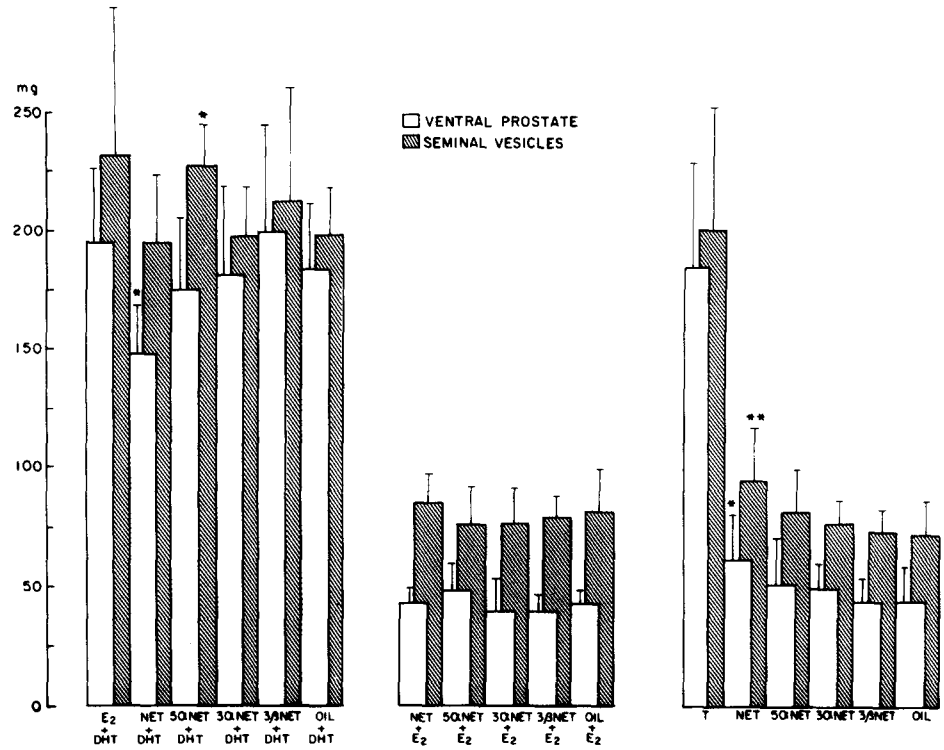


FIG. 2. Peripheral androgenic and antiandrogenic effects of norethisterone (NET) and its metabolites in long-term castrated male rats. Effect of daily treatment for 21 days with NET or its A-ring reduced derivatives (300 μg) in combination with either dihydrotestosterone (DHT, 300 μg) or estradiol ($E_2$, 5 μg), or given alone (500 μg) on accessory sex organs growth. Castrated animals treated with DHT (300 μg) plus $E_2$ (5 μg), DHT (300 μg) plus vehicle (10% ethanol-corn oil), $E_2$ (5 μg) plus vehicle, testosterone (T, 500 μg), or vehicle served as controls.

TABLE 2

PARAMETERS OF SEXUAL ACTIVITY DISPLAYED BY CASTRATED MALE RATS UNDER THE VARIOUS DAILY STEROID TREATMENTS

| | Vehicle+$E_2$[a] (n=8) | DHT[b]+$E_2$[a] (n=8) | NET[b]+$E_2$[a] (n=9) | 5α-NET[b]+$E_2$[a] (n=10) | 3α,5α-NET[b]+$E_2$[a] (n=9) | 3β,5α-NET[b]+$E_2$[a] (n=10) |
|---|---|---|---|---|---|---|
| % Ss With Mount | 100 | 100 | 100 | 100 | 100 | 100 |
| % Ss With Intromission | 63 | 100 | 89 | 90 | 56 | 60 |
| % Ss With Ejaculation | 13 | 100‡ | 67* | 10 | 11 | 30 |
| % Tests With Mount | 79 | 96† | 73 | 81 | 63 | 74 |
| % Tests With Intromission | 25 | 77‡ | 44* | 34 | 16 | 27 |
| % Tests With Ejaculation | 2 | 63‡ | 22‡ | 3 | 8 | 10 |
| Interintromission Interval (sec)[c] | 126 ± 53 | 59 ± 17 | 104 ± 72 | 384 ± 308 | 75* | 135 ± 98 |
| Hit Rate[c] | 0.35 ± 0.21 | 0.70 ± 0.15 | 0.46 ± 0.23 | 0.23 ± 0.15 | 0.23 ± 0.26 | 0.32 ± 0.22 |
| No. Intromissions Preceding Ejaculation[c] | 20.00 | 12.80 ± 2.20 | 16.70 ± 2.90 | 13.00 | 12.00 | 12.70 ± 7.40 |
| Mount Latency (sec)[c] | 70 ± 127 | 14 ± 12 | 30 ± 17 | 30 ± 43 | 83 ± 81 | 72 ± 115 |
| Intromission Latency (sec)[c] | 66 ± 74 | 127 ± 157 | 155 ± 113* | 175 ± 164 | 153 ± 140 | 185 ± 350 |
| Ejaculation Latency (sec)[c] | 1740 | 839 ± 304 | 1263 ± 507 | 850 | 736 | 1073 ± 195 |
| Post Ejaculatory Interval (sec)[c] | 520 | 468 ± 83 | 597 ± 81 | 514 | 400 | 564 ± 90 |

[a]5 μg.
[b]300 μg.
[c]Mean ± SD.
*$p<0.05$; †$p<0.01$; ‡$p<0.001$ as compared with the vehicle+$E_2$-treated control group.

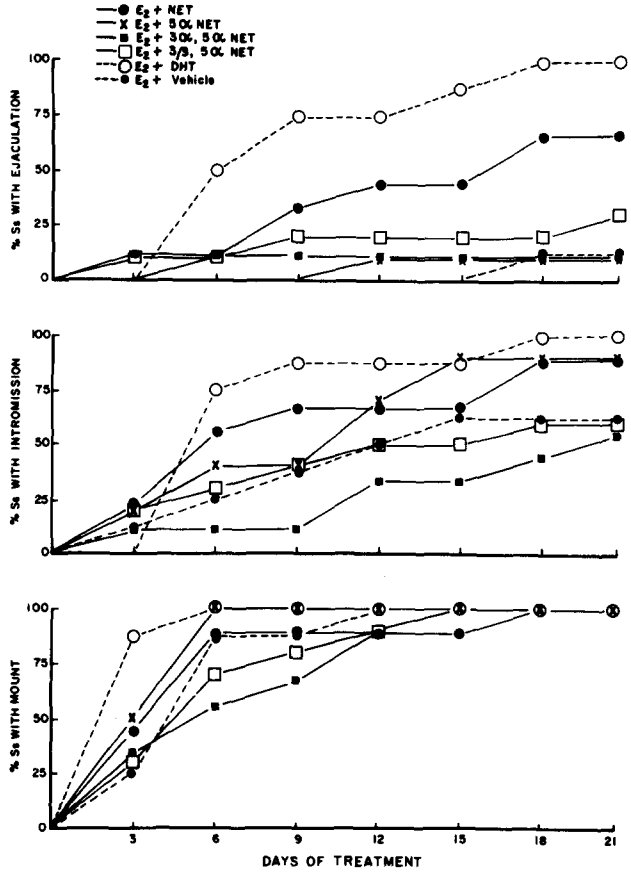


FIG. 3. Androgen-like behavioral effects of norethisterone (NET) and its metabolites in long-term castrated male rats.Cumulative percentage of castrated rats showing mounting, intromission, and ejaculation behavior during 21 days of treatment with NET or its A-ring reduced derivatives (300 μg) in combination with estradiol ($E_2$, 5 μg). Castrated animals treated with dihydrotestosterone (DHT, 300 μg) plus $E_2$ (5 μg) or with vehicle (10% ethanol-corn oil) plus $E_2$ (5 μg) served as controls.

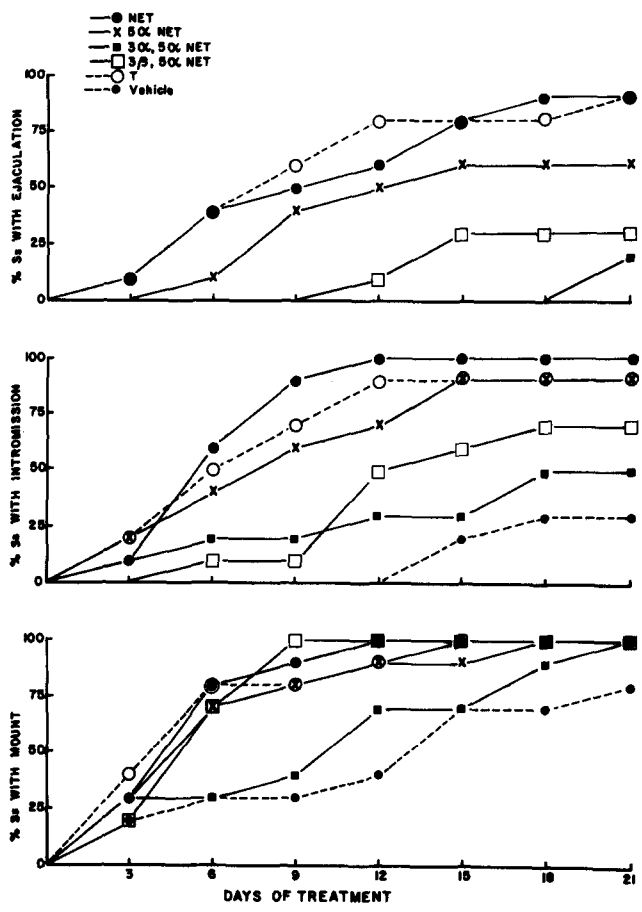


FIG. 4. Effectiveness of norethisterone (NET) and its metabolites to restore copulatory behavior in long-term castrated male rats. Cumulative percentage of castrated rats showing mounting, intromission, and ejaculation behavior during 21 days of treatment with NET or its A-ring reduced derivatives (500 μg). Castrated animals treated with either testosterone (T, 500 μg) or the vehicle (10% ethanol-corn oil) served as controls.

mean ± SD) and the ejaculation latency (857 ± 350 sec) were reduced to a level identical to that observed in the DHT (300 μg/day) plus $E_2$- (5 μg/day) treated group. No effect of NET and its derivatives on the ventral prostate and seminal vesicles weight was observed when administered with $E_2$ as compared with the control group (Fig. 2).

When unmodified NET was administered alone at a daily dose of 500 μg, it successfully elicited a complete copulatory pattern in a manner identical to that observed after the administration of testosterone at a similar dose level (Fig. 4, Table 3). Lower doses were ineffective (data not shown). Treatment with 5α-NET also restored male sexual behavior in the castrated animals, although to a lesser extent. The tetrahydro reduced derivatives of NET were poor inductors of male sexual behavior when administered alone (Fig. 4). NET-treated animals displayed sexual activities in a proportion of tests similar to that exhibited by the testosterone-treated group (Table 3). Rats receiving 5α-NET alone showed copulation at a lower level than testosterone-treated animals; however, sexual activity was displayed in a higher proportion of tests than that of oil-treated subjects. Administration of NET mimicked indeed the testosterone actions, particularly those of the mount latency and the number of intromissions preceding ejaculation, while 5α-NET administration exerted more limited effects. The 3β,5α-NET-, and to a lesser extent the 3α,5α-NET-treated animals, presented some mating activities considered to be estrogen-dependent (mount latency and the percent of tests with mount), but full copulatory behavior was not restored. A slight though significant increase on the ventral prostate ($p<0.05$) and seminal vesicles ($p<0.01$) weight was noticed in the group of animals receiving NET as compared with the oil control group, but the values were significantly lower than those of testosterone-treated animals (Fig. 2). A-ring reduced NET derivatives did not induce changes on male sex accessories.

## DISCUSSION

The results from this study gave evidence that the integrated analysis of male copulatory behavior in rodents is a suitable screening procedure to evaluate the hormone-like activities of synthetic progestins and their metabolic conversion products. The restoration of copulatory activity of the long-term castrated male rat was used to assess the estrogenic and androgenic potencies of NET and three of its neutral derivatives; the results also provided information on the mode of action of this progestin at the CNS.

The first set of experiments was aimed at elucidating the in-

TABLE 3

PARAMETERS OF SEXUAL ACTIVITY DISPLAYED BY CASTRATED MALE RATS UNDER THE VARIOUS STEROID TREATMENTS (500 μg/DAY FOR 21 DAYS)

| | Vehicle (n = 10) | Testosterone (n = 10) | NET (n = 10) | 5α-NET (n = 10) | 3α,5α-NET (n = 10) | 3β,5α-NET (n = 10) |
|---|---|---|---|---|---|---|
| % Ss With Mount | 80 | 100 | 100 | 100 | 100 | 100 |
| % Ss With Intromission | 30 | 90† | 100† | 90† | 50 | 70 |
| % Ss With Ejaculation | 0 | 90‡ | 90‡ | 60† | 20 | 30 |
| % Tests With Mount | 23 | 75‡ | 70‡ | 74‡ | 47† | 71‡ |
| % Tests With Intromission | 4 | 63‡ | 56‡ | 44‡ | 16* | 26‡ |
| % Tests With Ejaculation | 0 | 54‡ | 40‡ | 30‡ | 3 | 7* |
| Interintromission Interval (sec)[a] | 729 | 55 ± 24 | 108 ± 40* | 75 ± 51* | 155 ± 104 | 144 ± 100* |
| Hit Rate[a] | 0.12 ± 0.06 | 0.68 ± 0.17 | 0.47 ± 0.16* | 0.39 ± 0.19* | 0.36 ± 0.24 | 0.28 ± 0.16* |
| No. Intromissions Preceding Ejaculation[a] | — | 12.60 ± 2.10 | 11.40 ± 2.50 | 11.00 ± 2.10 | 15.00 | 18.70 ± 9.00 |
| Mount Latency (sec)[a] | 429 ± 330 | 30 ± 46 | 30 ± 36* | 50 ± 41 | 111 ± 113 | 66 ± 63* |
| Intromission Latency (sec)[a] | 490 ± 325 | 30 ± 47 | 91 ± 88 | 147 ± 154 | 368 ± 268 | 137 ± 99 |
| Ejaculation Latency (sec)[a] | — | 596 ± 207 | 1069 ± 336 | 580 ± 258 | 1580 | 1138 ± 410 |
| Post Ejaculatory Inverval (sec)[a] | — | 421 ± 94 | 607 ± 130 | 447 ± 89 | 840 | 485 ± 82 |

[a]Mean ± SD.

*$p<0.05$; †$p<0.01$; ‡$p<0.001$ as compared with the vehicle-treated control group.

trinsic estrogenic potency of these synthetic steroids by giving them simultaneously with DHT. The results clearly demonstrated that 3β,5α-NET, at the daily dose of 300 μg, induced a full male sexual behavior pattern with an incidence of copulatory events identical to that obtained following the administration of $E_2$-DHT used as the control (Fig. 1, Table 1). The observation that 3β,5α-NET specifically interacts with high affinity estradiol binding sites (9) strongly suggests that its behavioral effects are mediated by the intracellular estradiol receptor. Additional support to the concept that this nonphenolic, nonaromatizable NET derivative is acting centrally as an estrogen agonist is furnished by the demonstration that at the same dose level (300 μg/day) it is capable of inducing the synthesis of estrogen-dependent progesterone receptors in peripheral estrogen-sensitive tissues (28). The combined administration of 3α,5α-NET with DHT also restored full sexual behavior in the castrated male rats, although its efficiency was lower than that of its 3β epimer. This finding fits well with its relative binding affinity to the estradiol receptor and with the expression of its estrogenic activity on peripheral tissues which are also lower than those of 3β,5α-NET (9,28). The capability of 5α-NET to restore male copulatory behavior, when administered with DTH, was even lower than that exhibited by the 3α,5α-NET derivative. Since 5α-NET does not bind to the estradiol receptor (9), perhaps requires its further biotransformation to the tetrahydro derivatives to express an estrogenic behavioral activity. Interestingly, NET when combined with DHT, induced very little, if any, male copulatory behavior in the castrated animals at the dose employed (300 μg/day). This observation is in line with the lack of binding affinity of this synthetic progestin to the estradiol receptor (9,27), and also with experimental evidences which indicate that the NET molecule does not undergo enzyme-mediated aromatization in the nonpregnant condition (5,6). This finding also seems to support the conclusion that 500 μg NET/day, but not 300 μg NET/day, appeared to generate levels of A-ring reduced metabolites which were sufficiently high to exert estrogen-like effects.

The assessment of the intrinsic androgenic potency of NET and its metabolites revealed that only unmodified NET was able to restore significant full copulatory behavior when administered with $E_2$ in long-term castrated animals (Fig. 3, Table 2), in a manner similar to that observed with the same dose of testosterone. This observation is in parallel with the well demonstrated high-affinity binding of NET to the intracellular androgen receptor (9). Interestingly, NET, when administered with DHT, did not display an androgen-like effect in peripheral target organs and by the contrary it exerted an antiandrogenic effect, at least at the dose of 300 μg/day (Fig. 2), confirming and extending previous observations which indicated that synthetic progestins may exhibit either androgenic, antiandrogenic, or synandrogenic effects as the result of their interactions with the androgen receptor (7,8). These varying responses are species-, organ- and dose-dependent.

The finding that 5α-NET at two dose levels (300 and 500 μg/day) failed to induce male sexual behavior when given with $E_2$, strongly suggests that 5α-reduction results in a significant diminution of the behavioral androgenic potency of NET, in spite of its specific high-affinity binding to the androgen receptor (9). This lack of androgenic potency of 5α-NET was also evident in male sex accessories (Fig. 2). Both, 3α,5α- and 3β,5α-NET derivatives exhibited a complete lack of behavioral androgenic effects when administered with $E_2$ (Fig. 3, Table 2), an observation which correlates with their lack of binding affinity to the androgen receptor (9). These tetrahydro NET derivatives exhibited neither androgenic nor antiandrogenic effects upon male sexual accessories (Fig. 2), demonstrating that further A-ring reduction of 5α-NET abolishes its androgenic biological potency.

The results of experiments in which NET and its metabolites were administered alone at the daily dose of 500 μg, revealed that only NET resembled testosterone in terms of the biological potency for restoring full sexual behavior at an identical dose (Fig. 4, Table 3). This observation demonstrates that the NET molecule possesses both androgenic and estrogenic behavioral potencies in a level similar to that of the testosterone molecule. However, it must be stressed that while testosterone undergoes aromatization, NET does not, thus suggesting that enzyme-medi-

ated formation of A-ring reduced derivatives plays an essential role on the expression of the estrogenic effect of NET. Accordingly, it can be proposed that the effect of NET on male sexual behavior is primarily exerted by its potent intrinsic androgenic activity, mediated by androgen receptors, coupled with a complementary estrogenic activity furnished by its 3β, 5α- and 3α,5α-tetrahydro metabolites, acting via estrogen receptors. Even more, 5α-NET does not appear to have an important role on mediating NET-induced male sexual behavior as it was evident following its administration either alone or in combination with $E_2$. The lack of restoration of sexual behavior observed in the castrated animals treated with either 3α,5α-NET or 3β,5α-NET was predictable because of their lack of androgenic intrinsic activity. Interestingly, our results agree with the data of McGinnis and Dreifuss (16) who have proposed that under physiological circumstances, testosterone rather than 5α-DHT, plays a major role in the regulation of masculine sexual behavior in the rat with a complementary effect of estradiol.

In contrast with its potent androgenic behavioral activity, NET exerted only slight androgenic effects on male sex accessories, which were not comparable to those observed in the testosterone-treated animals (Fig. 2). A-ring reduction of the NET molecule abolished its androgenic activity at peripheral organs.

Taken together, these results strongly suggest that aromatization is not an essential step in the restoration of full copulatory behavior in castrated male rats following the administration of norethisterone. The data reported herein are consistent with the notion that pharmacological activation of mating requires a potent androgenic effect with a concomitant estrogen-like action, and this synthetic progestin fulfills both hormonal requirements. Indeed, evidence was given for 1) the intrinsic androgenic potency of NET and 2) the estrogen-like effects of its tetrahydro metabolites. Further studies are needed to determine whether under normal physiological conditions, steroid hormone control of masculine sexual behavior in male rats may require a similar mechanism of action as that being proposed to mediate the behavioral actions of NET, a synthetic 19-nor steroid.

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