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Androgens Are Specifically Implicated in Female Rat Sexual Motivation. The Influence of Methyltrienelone (R1881) on Sexual Orientation

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DE JONGE F H E H KALVERDIJK AND N E VAN DE POLL *Androgens are specifically implicated in female rat sexual motivation The influence of methyltrienelone (R1881) on sexual orientation* PHARMACOL BIOCHEM BEHAV **24**(2) 285–289 1986 —The present experiment was designed to investigate whether androgens are specifically involved in the induction of a male-directed orientation in adult female rats Ovariectomized female rats were either treated with the non-aromatizable androgen methyltrienelone (R1881), with testosterone propionate (TP), with estradiol benzoate (EB) or with an equal volume of the solvent Sexual orientation of these females towards either sexually active males or estrous females was then investigated and related to levels of receptive and mounting behavior Compared to the solvent-treated females females treated with R1881, TP or EB spent more time near sexually active males Mounting behavior was stimulated in the R1881- and TP-treated females, but EB-treated females mounted as often as females treated with the solvent only Lordosis behavior was only observed in TP-treated or EB-treated females Mount frequency of the females of the different treatment groups was positively correlated with time spent near males These correlations reached statistical significance in the TP-treated and EB-treated females In the TP-treated females, the lordosis quotient was negatively correlated with time spent near males The results of the present experiment suggest that androgens need not be converted into estrogens in order to facilitate a male-directed orientation in ovariectomized female rats

R1881 EB TP Sexual orientation Female rats

SEVERAL experiments have indicated that estrogen- (E) or testosterone- (T) treated female rats will spend more time near sexually active male incentives than untreated controls, when given the choice to orient towards male or female incentives [8, 22, 23 24] These experiments also indicated that the increased interest is sexual rather than social and they suggest that increased levels of receptive behavior in reaction to male mounting are generally accompanied by a male-directed sexual orientation [21]

Stimulation of receptive behavior after androgenic stimulation is generally thought to be mediated by estrogenic metabolites [14,20] derived from T after aromatization, a process which has been shown to occur in the brains of all mammalian species studied thus far [17, 18, 27] It remains still to be investigated, however whether conversion from T to E is required for the induction of a male-directed orientation as well It has previously been suggested that androgens in particular are involved in (hetero-) sexual motivation of female rats [8], monkeys [11, 12, 16, 26] and also in humans [3, 13, 35], although others have presented evidence to the contrary [2,15] In female rats, the hypothesis that aromatization from T to E is required for androgen induced (hetero-) sexual motivation could not be unambiguously confirmed [19] These authors investigated effects of dihydrotestosterone (DHT) and effects of the estrogen antagonist MER25 on E- or T-activated sexual motivation in the female rat Although DHT was shown to be ineffective with respect to the stimulation of a male-directed orientation in female rats, the estrogen antagonist MER25 could only delay, but not abolish, androgen induced motivation to approach sexually active male rats

Recently, methyltrienelone (R1881) has been developed as a synthetic, non-aromatizable androgen [1, 5, 6, 9, 10] which was shown to stimulate mounting, but not receptive behavior in the ovariectomized female rat [25] The present experiment was designed to determine the effects of R1881 on sexual orientation of the ovariectomized female rat in order to test whether or not androgens are involved in female sexual motivation Sexual orientation was measured in a semi-open field apparatus [7,23] in which female rats are given the choice to orient either towards sexually active males or towards estrous females Under these circumstances, T or E have previously been shown to induce a male-directed orientation in female rats [8,22] In the present experiment, effects of R1881 on sexual orientation were compared to those of testosterone propionate (TP), estradiol benzoate (EB) and treatment with the solvent only Receptive and mounting behavior were observed after the tests for sexual orientation It was postulated that R1881 would stimulate a male-directed orientation, because it has been

suggested that androgens are specifically involved in female sexual motivation [8] Previous experiments at our lab, however, have shown that manipulations which increase mounting may result in a female- instead of a male-directed orientation (de Jonge, Burger, van Haaren, Overdijk and van de Poll, submitted) Since R1881 has been shown to stimulate mounting, but not receptive behavior [25], it might, as an alternative hypothesis, be predicted that R1881 will stimulate orientation towards an estrous female

METHOD

Animals and Hormone Treatment

Female Wistar rats (n=72, 180–200 gram) obtained from the animal supply house of TNO (Zeist, the Netherlands) were ovariectomized under fentanyl anesthesia (hypnorm 0 1 ml/rat, 0 02%) on arrival at the laboratory and left undisturbed for two weeks before experimentation They were housed in macrolon cages containing 6 animals per cage under a reversed dark-light cycle (lights off 2 30 a m –2 30 p m) Food and water were ad lib available

Ovariectomized stimulus females and stimulus males of proven sexual vigour were used as incentives during partner preference tests They also served as stimuli to elicit mounting and receptive behavior during sexual interactions According to standard procedures at our laboratory [34], stimulus females were artificially brought into heat by 50 μg EB (estradiol benzoate, 48 hr prior to testing) and 1 mg progesterone (17 hr prior to testing) Stimulus females were found to be highly receptive and proceptive at the time of testing

Doses of testosterone propionate (TP, 250 μg) and estradiol benzoate (EB, 4 μg) were selected on basis of previous investigations indicating that these doses are effective with respect to the stimulation of receptive behavior and with respect to the induction of a male-directed orientation [8,24] The synthetic androgen methyltrienelone (R1881) was previously found to be equally potent as T in stimulating masculine sexual behavior in male rats [33] and was therefore also injected in a dose of 250 μg TP (250 μg), R1881 (250 μg) and EB (4 μg) were dissolved in a suspension of propylene glycol and oil (PG, 125 1) Daily intra-muscular injections (0 1 ml per rat) were given

Procedure

At the start of the experiment females were divided into 4 groups of 18 animals each Each group was injected daily with either 250 μg R1881, 250 μg TP, 4 μg EB, or an equal volume (0 1 ml) of the solvent (PG) After 10 days of hormone treatment, females were tested for sexual orientation on three consecutive days The females were then left undisturbed for one day and were subsequently tested for mounting as well as receptive behavior on two consecutive days On both of these days, females were first tested for mounting behavior and then for receptive behavior one hour later Testing took place under dim red light illumination and during the last quarter of the subjects' dark hours

Tests for Sexual Orientation

These tests were run in semi-open field cages [7,23] consisting of an open field arena (80×80×35 cm) with two small boxes (15×12×12 cm) positioned opposite to each other, in which stimulus animals could be placed A gauze partition separates these animals and the experimental animal, allowing both animals to see and smell each other without physical contact Small areas (25×25 cm) in front of the stimulus compartments were balanced upon microswitches which activated electronic counters to record frequencies and duration of visits of the experimental animals to the stimulus animals In the present experiment the two incentives were always a sexually active stimulus male and a stimulus female artificially brought into heat (see animals and hormone treatment) The relative position of these incentives was randomly varied between subjects to correct for possible position effects Tests lasted 15 minutes and were started by placing a female in the middle of the semi-open field After each test the apparatus was thoroughly cleaned

Tests for Sexual Behavior

Tests were run in semi-circular cages (r=25 cm) with sawdust-covered floors

Tests for Mounting Behavior

Experimental females were allowed to adapt to the test environment for 5 minutes before a stimulus female was dropped into the test cage During the following 20 minutes latency to the first mount and mount frequency were scored Mounts were only counted as such when accompanied by pelvic thrusting Intromission patterns were included in the total mount score

Tests for Receptive Behavior

Stimulus males were allowed to adapt to the test environment for 5 minutes Thereafter, the experimental female was dropped into the test cage and lordosis behavior was scored The lordosis quotients (LQ) were then calculated from the number of lordoses shown in reaction to male mounting (LQ=number of lordoses/number of mounts × 100%) The stimulus male was allowed to mount 6 times In addition, females were designated as "proceptive" when hopping, darting or ear wiggling was observed

Statistics

Analysis of the results revealed considerable inter individual variance, especially in mounting behavior Results were therefore analysed by non-parametric statistics [28]

RESULTS

Tests for Sexual Orientation

Figure 1 shows the percentage of testing time spent near males and females respectively, as an average of the three consecutive tests

Non-parametric analysis of variance (Kruskal-Wallis tests) over the four different treatment groups indicated that the groups differed with respect to time spent near males ($p<0$ 005), but not with respect to time spent near females ($p>0$ 82) Subsequent inter group comparisons indicated that time spent near males was significantly greater in the R1881-treated (Mann-Whitney, U=82 0, $p<0$ 05) and EB-treated females (U=43 0, $p<0$ 001) as compared to the PG-treated females TP-treated females also spent more time near males (27 5% testing time) than the PG-treated females (22 4% testing time), but the difference was only marginally significant (U=97 0, $p<0$ 06) Time spent near males was not significantly different among the R1881-treated, TP-treated or EB-treated females (U>107 0, $p>0$ 2)

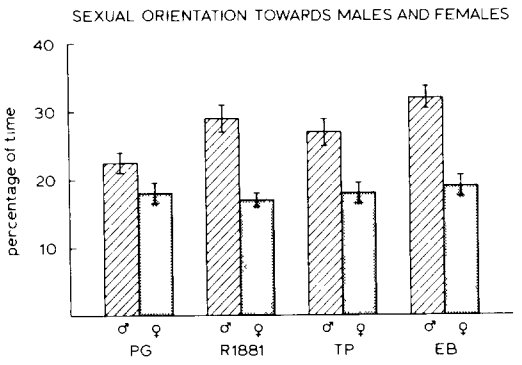

FIG 1 The percentage of testing time (including Standard Errors of the Mean) spent near sexually active males and estrous females The four different treatment groups (all groups, n=18) are presented on the abscissa PG=propylene glycol, R1881=methyltrienelone, TP=testosterone propionate, EB=estradiol benzoate

Within group analysis of preference behavior revealed that females of all four treatment groups spent more time near the sexually active male than near the estrous female (Sign-test, $p<0.05$ for all groups) The four treatment groups differed, however, when sexual orientation behavior during the three consecutive test days was analysed Table 1 presents the number of females which spent more time near the female or near the male on three consecutive test days The other experimental females were not consistent in their preference behavior

The number of females which consistently preferred the company of the male as opposed to those which did not show consistent preference behavior was significantly greater in the R1881- (Chi-square, $p<0.05$), TP- (Chi-square, $p<0.05$) or EB-treated females (Chi-square, $p<0.05$) than in the PG-treated females There were no differences between R1881-, TP- or EB-treated females (Chi-square, $p>0.6$ for all inter group comparisons)

Tests for Sexual Behavior

Results on mounting and lordosis behavior were averaged over the two consecutive test days They are presented in Fig 2 for the different treatment groups

Analysis of variance by Kruskal-Wallis tests revealed a significant effect of hormonal treatment on mount frequency ($p<0.0001$) Subsequent inter group comparisons indicated that R1881-treated ($U=77.5, p<0.01$) and TP-treated females ($U=53.5$, $p<0.001$) mounted more frequently than PG-treated females Mount frequency was not facilitated in the EB-treated females ($U=152.5, p>0.7$) Mount frequency of the R1881-treated females was not different from mount frequency of the TP-treated females ($U=136.5$, $p>0.4$) Analysis of variance by Kruskal-Wallis tests also revealed a significant effect of hormonal treatment on lordosis quotients ($p<0.0001$) Lordosis behavior was never observed in the PG or R1881-treated females TP- and EB-treated females showed a higher lordosis quotient (LQ) than PG-treated females ($U=0.0, p<0.001$ for both groups) LQ of the EB-treated females was significantly higher than that of the TP-treated females ($U=0.0, p<0.001$) Proceptive behavior was observed in 22.2% of the TP-treated and 100% of the EB-treated females, but never in the PG- or R1881-treated females

TABLE 1

PREFERENCE BEHAVIOR

Preference on three consecutive test days	PG	R1881	TP	EB
for a female	1	0	0	0
for a male	3	11	10	9
not consistent	14	7	8	7
total	18	18	18	16

Number of females showing the same preference behavior on three consecutive test days

Preference = time spent near incentive$_1$ > time spent near incentive$_2$

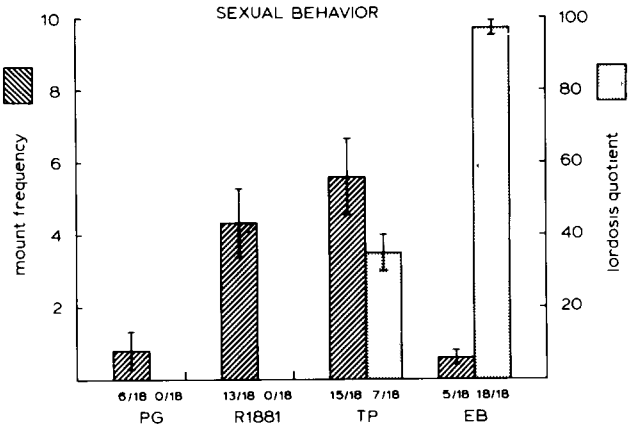

FIG 2 Sexual behavior of the females whose sexual orientation is presented in Fig 1 For each treatment group, average mount frequency (including Standard Errors of the Mean) is presented at the left and lordosis quotients (LQ, including Standard Errors of the Mean) are presented at the right Number of responding females (out of 18) is presented at the bottom of each bar For abbreviations, see Fig 1

Correlations Between Sexual Behavior and Sexual Orientation

Spearman rank coefficients of correlation were calculated for masculine and feminine sexual behavior (averaged over two test days) on the one hand and sexual orientation (i e , time spent near males, resp time spent near females, averaged over three test days) on the other hand Mount frequency was never significantly correlated with time spent near females ($-.15<r<.13, p>0.32$) Mount frequency was positively correlated with time spent near males in the TP- and EB-treated females ($r>.44, p<0.01$), but correlation coefficients were not statistically significant in the PG- or R1881-treated females ($0<r<.17, p>0.32$)

Correlation coefficients between LQ and sexual orientation were only calculated for the TP-treated females, since LQ's were either 0 or 100 percent in the remaining treatment groups LQ was negatively correlated with time spent near males in the TP-treated females ($r=-.75, p<0.05$)

DISCUSSION

The present experiment has shown that the synthetic, non-aromatizable androgen R1881 is equally effective as EB or TP in inducing a male-directed orientation in ovariectomized female rats In EB-treated females, this male-directed orientation was accompanied by lordosis quotients of nearly 100% R1881-treated females, however, never showed lordosis behavior When females were treated with TP, the male-directed orientation was accompanied by lordosis quotients of about 54% In these females lordosis behavior was negatively correlated with time spent near males

The present results confirm previous observations on TP- or EB-induced sexual orientation towards males [8, 22 23, 24], and on the absence of feminine sexual responses and facilitation of mounting behavior in female rats which are treated with R1881 [25] Contrary to other experiments [8,22], a slight preference for the sexually active male incentive was observed when females were treated with the solvent only Possibly as a result of this, the increased interest for a male as observed in the TP-treated females was only marginally significant Analysis of choice consistency, however, indicated that the hormonally manipulated females consistently preferred the male, while solvent treated females did not Results on consistency of preference behavior should be taken as additional evidence that TP, as well as EB and R1881, stimulated interest for a male in the present experiment This observation corroborates results of previous experiments [8, 22, 24, 32]

One of the predictions in the introduction was that R1881-treated females would orient towards sexually active males, since androgens have been specifically implicated in (hetero-) sexual motivation of female rats [8], monkeys [11, 12, 16, 26] and humans [3, 13, 35] The results of the present experiment support this hypothesis R1881 has been shown to bind to putative intracellular androgen receptors with an affinity higher than that of DHT [1, 5, 6, 10] In addition, R1881 is thought not to be aromatized to estrogens in peripheral systems [31], nor does it bind to estrogen receptors in the brain [29,30] Given these properties, our results on the synthetic androgen R1881 clearly show that aromatization from T to E is not necessary for the induction of a male-directed orientation in female rats However, TP and EB were just as effective as R1881 in inducing a male-directed orientation, and it is therefore likely that estrogenic metabolites of T also contribute to the TP induced male-directed orientation The suggestion that both androgens and estrogens are involved in TP-induced male-directed orientation is supported by data indicating that the estrogen antagonist MER25 completely inhibited EB-induced (hetero-) sexual motivation of female rats, while MER25 only delayed, but not completely inhibited, the TP-induced response [19]

The results of the present experiment, which support the hypothesis that androgens are specifically involved in (hetero-) sexual motivation of female rats, are somewhat contradictory to the observation that DHT, the 5α-reduced, non-aromatizable androgenic metabolite of testosterone, was not effective in facilitating (hetero-) sexual motivation in female rats [19] However, the ineffectiveness of DHT (as compared to T) in stimulating an androgen dependent behavior does not necessarily imply that T has to be aromatized to E in order to stimulate this behavior Sodersten and Gustafsson [33], for instance, have shown that DHT was ineffective in stimulating male sexual behavior in male rats, while R1881 was shown to be highly effective in this respect The latter observation led Sodersten and Gustafsson to suggest that rapid metabolisation of DHT could explain the relative ineffectiveness of DHT as compared to R1881 or T

Data obtained so far suggest that increased levels of receptive behavior in the female rat are generally accompanied by an increased interest for a sexually active male incentive [21] Although our results on EB-treated females confirm this notion, the present results suggest that the inverse may not be necessarily true (i e , increased interest for a sexually active male needs not to be accompanied by increased levels of receptive behavior) The R1881-treated females did not show any receptive behavior, while time spent near males was negatively correlated with receptive behavior in TP-treated females The results therefore indicate that hormonal mechanisms underlying the facilitation of receptive behavior are clearly dissociated from those underlying sexual orientation towards a male

In the present experiment, sexual orientation was measured in a situation where sexual interaction was not possible Obviously, therefore, the present test situation prohibits definite conclusions as to whether motives underlying the male-directed orientation were sexual or social in nature It was shown in experiments in our laboratory that TP-treated females prefer the company of sexually active males when given the opportunity to obtain sexual reward, while EB was less effective in this respect [32] It seems therefore likely that the male-directed orientation, at least in the TP-treated females is sexual in nature R1881-treated females, however, do not show any receptive behavior when confronted with a male It seems therefore difficult to establish whether R1881 would stimulate females to seek sexual reward, unless additional hormonal treatment is given which renders them receptive

Male-directed orientation in R1881- or TP-treated females was accompanied by high levels of mounting behavior in the present experiment It has previously been suggested that mounting in the female rat may be indicative of high sexual motivation (i e , an increased interest for a sexually active male incentive) [4,36] Conversely, other observations suggest that mounting may be related to an increased interest for an estrous female [8] When female rats were repeatedly given the opportunity to mount other females, they showed a female-directed orientation when tested in a situation in which sexually naive female rats showed a male-directed orientation (de Jonge *et al* , submitted) In the present experiment, mounting behavior was positively correlated with the time spent near sexually active male incentives, the correlations being significant in the EB- and TP-treated females The data therefore support the idea of mounting being indicative of a high (hetero-) sexual motivation in female rats In contrast, no indication could be found for a relationship between mounting and a female-directed orientation, even not in the R1881-treated females, which showed high levels of mounting behavior and no receptive behavior It is therefore suggested that mounting behavior in female rats can be taken as an indication of general sexual arousal, which is accompanied by a male-directed orientation in sexually naive female rats and by a female-directed orientation when females are sexually experienced with respect to mounting (de Jonge *et al* , submitted)

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