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Genetic Differences in Female House Mice in Aggressive Response to Sex Steroid Hormone Treatment

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COMPAAN, J. C., G. VAN WATTUM, A. J. H. DE RUITER, G. A. VAN OORTMERSSEN, J. M. KOOLHAAS AND B. BOHUS. *Genetic differences in female house mice in aggressive response to sex steroid hormone treatment.* PHYSIOL BEHAV **54**(5) 899–902, 1993.—Male mice, genetically selected for aggression, characterized by short attack latency (SAL) or long attack latency (LAL), differ on several testosterone (T)-related parameters during ontogeny and adult age. The variation in aggressive behavior at adult age may be due to differences in degree of androgenization prenatally. When exposed to T at prenatal, neonatal, and/or adult age, nonlactating females also display intraspecific fighting behavior. In the present study, we investigated in females of the SAL and LAL selection lines, whether the differentiation of aggression involves processes similar to ones seen in males. Therefore, we injected females with testosterone propionate (TP) or vehicle on the day of birth, treated them after ovariectomy at adult age with T, estradiol (E), or vehicle, and tested their aggressive response. We found that neonatally vehicle-treated SAL females show a higher aggressive response to chronic T treatment at adult age than LAL females receiving the same treatment. Females of both selection lines treated with vehicle or E as adults were not aggressive. Neonatal TP treatment did not influence the adult T sensitivity and difference between selection lines in response to T at adult age. However, neonatally TP-treated SAL females showed aggressive behavior when treated with E at adult age, whereas LAL females failed to do so. These results suggest a genetic difference in susceptibility to T and E, which plays a major role prenatally, in organizing the development of sex steroid-dependent neural systems.

Testosterone Estradiol Ontogeny Aggression Female mice Genetic differences

CHRONIC testosterone (T) treatment induces aggression at adult age in mice and rats, both in males and nonlactating females [(2,27,29); for review see (1,6)]. The latter generally do not display spontaneous intraspecific fighting behavior. In addition, neonatal androgen exposure in females increases the adult aggressive response to T. Neonatally androgenized females begin to fight much sooner following the start of hormone treatment in adulthood than do neonatally vehicle-treated mice (33,38). Accordingly, neonatal exposure to testosterone seems to sensitize the central nervous system to the aggression-eliciting property of T (7,12,36). Also, prenatal exposure to androgens facilitates adult aggressive response to T (15,19,34). Such prenatal T exposure may originate from the uterine position of females with respect to male fetuses (14,20).

In our laboratory, two selection lines of wild mice (*Mus musculus domesticus*), one for short attack latency (SAL) and one for long attack latency (LAL), and a randomly bred control line are available (5,30,31). Both adult and perinatal levels of T play an important role in the expression of adult intermale aggressive behavior in these genetic selection lines. We found that adult males of the aggressive SAL line have a higher plasma T level, a higher seminal vesicle weight (32), and a larger testicular T production capacity (10) compared to mice of the nonaggressive LAL line. Furthermore, prepubertally castrated SAL males show a higher aggressive response following adult T replacement than similarly treated LAL animals, suggesting a higher T sensitivity of the central nervous system (32). However, neonatally the LAL males have a larger testicular T production capacity than SAL males at the same age (10). Moreover, after neonatal testosterone treatment, LAL males showed a further reduction in aggressive behavior at adult age, whereas aggression of SAL males was not affected (9).

To investigate whether similar processes are involved in the differentiation of female aggression, we tested females of these selection lines to determine if they differ in aggressive response to the same neonatal and/or adult testosterone treatment. Therefore, we injected female mice of both selection lines with testosterone or vehicle on the day of birth, treated them after ovariectomy at adult age chronically with T, estradiol, or vehicle, and tested their aggressive response.

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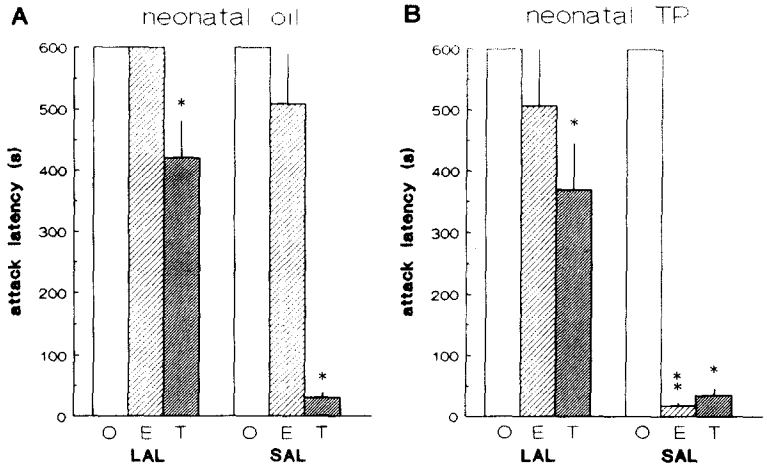

FIG. 1. Mean attack latencies (ALS + SEM) of females of the short attack latency line (SAL) and long attack latency line (LAL) neonatally treated with vehicle (oil) (A) or testosterone propionate (B) and chronic sex steroid treatment at adult age. Open bars: oil (O); lightly hatched bars: estradiol (E); heavily hatched bars: testosterone (T). Both neonatally oil- and TP-treated SAL and LAL females show a significant effect of chronic T treatment at adult age (*$p < 0.05$), in which the SAL females show a higher aggressive response than LAL females ($p < 0.01$) (A and B). Only the neonatally TP-treated SAL females show an aggressive response to adult E treatment (**$p < 0.01$), which significantly differs from similarly treated LAL females ($p < 0.05$) (B).

METHOD

Animals

Female mice (*Mus musculus domesticus*) from selection lines bred in our laboratory were used. These lines have been genetically selected for either long intermale attack latency (LAL; generations 14–17) or short intermale attack latency (SAL; generations 37–39) (30,31). Parents were housed on a sawdust bedding in standard mouse breeding Plexiglas cages (17 × 11 × 13 cm), in animal rooms with controlled light/dark cycle (12:12; lights off at 1230 h) and temperature (19–21°C). Standard lab chow and water were available ad lib.

On the morning after the night of birth (day 1), all pups were treated with testosterone or vehicle. At 3 weeks postpartum, the young animals were separated from the parents and were kept apart according to sex. At an age of 10 weeks, after ovariectomy (OVX), the females were housed individually in standard cages (17 × 11 × 13 cm). Aggressive behavior was tested at the age of 14 weeks. Because normal female mice require a long exposure to testosterone to display aggression (27,33,38), the females received the hormone for 4 weeks.

Treatment

On day 1, between 1100 and 1200 h, a complete litter was injected subcutaneously (SC) in the neck with either 3.24 μg testosterone propionate (TP) dissolved in 20 μl oil (Organon, Neo-Hombreol U.R. RVG00045) or just peanut oil. Prior to the aggressive behavior test, females were ovariectomized (OVX) at the age of 10 weeks, immediately followed by an implantation of either a standard testosterone (T) pellet, an estradiol (E) pellet, or one containing oil. The surgery was carried out under ether anesthesia and the pellet was implanted SC in the neck. The pellet consisted of a silastic tube (Rubber B.V., python; length 12 mm; i.d. 1.0 mm; o.d. 3.0 mm) filled with either peanut oil, 5.0 mg of estradiol (Organon, oestradiol micron-Oes Mic 2004) (23), or 1.0 mg of testosterone (Organon, testosterone micron-Te Mic 2001) (1) and dissolved in oil. Both sides of the tube were sealed with a Silicon glue. Pellets were stored in the dark at 4°C. During the night before implantation, the pellets were kept in saline (0.9 % NaCl solution) to initiate diffusion. The neonatal and adult treatment yielded six groups (oil-oil, oil-E, oil-T, TP-oil, TP-E, and TP-T, n = 6–9) of females in both selection lines.

Aggressive Behavior Test

Four weeks after surgery, the aggressive response was tested. As a measure of aggressive behavior, the attack latency score (ALS) was used (30). The ALS is the mean of the latencies to attack an opponent on 3 consecutive days, determined between 1300 and 1500 h, during the dark phase. Each experimental female was confronted with a standard, gonadally intact male albino mouse (MAS-GRO). The test was terminated immediately after the experimental female attacked the opponent, or, in the absence of any attack, after 10 min. Thus, for those animals that did not fight at all the ALS was set at 600 s.

Statistics

Because the ALS data were not normally distributed, significant neonatal and adult treatment effects between groups were determined with nonparametric Mann–Whitney U-test.

RESULTS

The mean attack latencies (ALS) of the experimental females of the short attack latency line (SAL) and long attack latency line (LAL) are presented in Fig. 1. When treated with vehicle (oil) neonatally [Fig. 1(A)], both SAL and LAL females displayed aggressive behavior towards a male opponent after a T pellet was implanted at adult age. These females differ significantly from the females with an oil or E pellet ($p < 0.05$). However, the attack latency score of SAL females (ALS = 30.9 ± 7.7 s) is significantly shorter than the ALS of LAL females (ALS = 420.3

± 60.4 s; $p < 0.01$). This means that SAL females display a higher aggressive response to the standard adult T treatment than the LAL females. Moreover, all SAL females of this group attacked the opponent, whereas only 63% of the LAL females showed fighting behavior. Females with an E or oil pellet did not attack the opponents. In fact, it was frequently observed that the opponents did attack the ovariectomized females provided with an oil pellet (data not presented).

A comparison between the neonatal oil treatment and the neonatal TP treatment revealed that neonatal TP does not affect the attack latency score in the adult oil-treated or in the adult T-treated females. As presented in Fig. 1(B), after neonatal TP treatment SAL females provided with a T pellet show a shorter attack latency score (ALS = 35.1 ± 11.2 s) than similarly treated LAL females (ALS = 369.2 ± 76.9 s; $p < 0.01$). Furthermore, the percentage of animals that fight did not change after a standard testosterone dose immediately after birth: all adult T-treated SAL females attacked the albino mice, whereas 56% of the adult T-treated LAL females displayed aggressive behavior.

However, all SAL females with an E pellet at adult age were highly aggressive if neonatally treated with testosterone [Fig. 1(B)]. The ALS of these neonatally testosterone-treated females is significantly shorter (ALS = 18.2 ± 4.0 s) than SAL females with neonatal oil injection (ALS = 600 s; $p < 0.01$). In contrast to these SAL-TP-E females, the LAL-TP-E females hardly show aggression towards an opponent (ALS = 506.7 ± 93.4 s; $p < 0.05$).

DISCUSSION

The results of this experiment revealed that females of both the aggressive SAL and nonaggressive LAL selection lines display fighting behavior towards a male opponent after chronic T exposure at adult age. This is in accordance with several reports on chronic T-induced aggression in nonlactating female mice (2,25,27) and rats (29). However, T-treated SAL females attack the male intruder of the territory much faster than the T-treated LAL females. This may indicate that the central nervous system (CNS) of SAL females is more sensitive to the aggression-promoting properties of T. One may argue that the duration of adult T exposure was not long enough to facilitate a shorter attack latency in LAL females. However, we would still deal with a difference in the length of hormone treatment until fighting behavior occurs. This would also indicate a variation in T sensitization. The difference in adult CNS T sensitivity is in accordance with data obtained from prepubertally castrated males of the same selection lines. The SAL males also show a much higher aggressive response to a standard T treatment at adult age than LAL-type males (32). Hence, the CNS of both males and females of the SAL selection line is more sensitive to the aggression-eliciting properties of T at adult age compared to the LAL line.

Earlier experiments in males indicated that neonatal testosterone is an important factor in the differentiation of aggressive behavior. However, in the present study, we found that neonatal TP treatment does not influence the adult attack latency after chronic T treatment in females of these selection lines. This indicates that the CNS sensitivity to T at adult age is not affected by neonatal TP treatment. However, we cannot exclude the possibility that neonatal TP has modified the duration of adult T exposure necessary to elicit aggressive behavior (33,38). Nevertheless, it can be concluded that neonatal TP treatment does not affect the difference in adult T-induced aggressive behavior between the two selection lines, neither in males nor females. Accordingly, the adult difference between SAL and LAL animals in neural testosterone sensitivity seems to be established before birth.

Several reports suggest that prenatal testosterone treatment influences the sensitivity of the CNS to the aggression-eliciting properties of T (15,19,34) and facilitates masculine sexual behavior (4,16) in later life. It is known in rats (37), mice (35), and ferrets (3) that before birth male fetuses produce more testosterone than female fetuses. Contiguity to male fetuses (14) or the presence of caudally located males within a uterine horn (20) induces partial masculinization of the female CNS. However, a more recent study by Gandelman et al. (13) showed that contiguity to male fetuses does not influence the display of heterotypic sexual behavior by female mice in response to T treatment. Others found strain differences in aggression of female mice treated with T at adult age, whereas no variation was found within a strain due to uterine position (8,22). Apparently, it is not just the circulating testosterone in utero, but a more general difference in genotype in interaction with prenatal T that plays a major role prenatally in the organization of adult CNS T sensitivity.

Surprisingly, all neonatally androgenized SAL females respond with a very fast attack of the male opponent after chronic E exposure, whereas the attack latency in similarly treated LAL females is not affected. Apparently, the neonatal TP treatment does influence the sensitivity to chronic E treatment at adult age. Neonatal T is necessary to induce fighting behavior after chronic E treatment in adulthood (17,23), whereas neonatally nonandrogenized females do not show aggressive behavior, even when given large amounts of E in later life (11,25,29). However, neonatally androgenized LAL females did not show E-induced aggressive behavior. Obviously, a clear strain difference exists in the capacity of neonatal T in sensitizing the adult brain to estradiol. Indeed, neonatal TP treatment induces stronger effects on aggression of intact females (not ovariectomized) of aggressive mice strains (21,28). Moreover, Simon and Whalen (24) showed, in males, a genetically determined difference between several strains of mice in sensitivity to the aggression-eliciting properties of androgens or estrogens. Accordingly, genetic differences in sensitivity to sex steroids play a major role in the variation of aggressive behavior.

The genetic variation in neonatal T sensitivity indicates a prenatal differentiation in the organization of sex steroid-dependent neural systems. According to the present study, one important factor involved in the differentiation of aggressiveness is the conversion of testosterone to estradiol by aromatase during ontogeny. In neonatal male rats a higher aromatase activity exists in pooled samples of several brain parts, i.e., hypothalamus, preoptic area, septum, and amygdala (18). It is possible that a variation in T level perinatally results in differences in aromatase activity at adult age (26).

In summary, females as well as males of the aggressive SAL selection line are more sensitive to the aggression-promoting property of testosterone at adult age than LAL animals, irrespective of neonatal TP treatment. Furthermore, only neonatally androgenized SAL females respond to adult chronic E exposure, whereas similarly treated LAL females are not affected. These results suggest a genetic difference, which plays a major role prenatally, in organizing the development of sex steroid-dependent neural systems. The involvement in this process of sex steroids, their converting enzymes, and sex steroid receptors during ontogeny should be investigated in the future.

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