GENETICS

Effect of Genotype on Behavioral and Hormonal Components of Sexual Activation of Male Mice

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Possible relationship between changes in behavior and hormone response to a sex stimulus was studied by comparing these parameters in mice of 10 genotypes. All males spent more time near the wall during the very first 5 min after appearance of the female. Strains with the minimum (DBA/2, C57Bl/6J) and maximum (SWR, CBA) time spent near the wall were distinguished. Genotypical differences in hormone response during sexual activation were detected. Considerable increase in testosterone concentration in the peripheral blood was observed in males of 6 of the 10 mouse strains 30 min after appearance of the female. Mouse strains with contrast sexual activation were distinguished. No correlation between behavioral and hormonal response was detected. The detected genetically determined variability of the behavioral and hormonal components of sexual activation is determined by hereditary peculiarities of central mechanisms regulating the studied functions.

Key Words: *mice; sexual activation; testosterone; genotype; genetic control*

Genetic regulation of the reproductive system is an important problem of behavioral genetics. Some individual parameters of this regulation can be evaluated in experiments on genetically homogeneous inbred animal strains. Differences in plasma testosterone concentration [2,5,7], an important parameters of male endocrine function, were demonstrated.

Sexual activation is the initial stage of sexual behavior triggering the entire complex of processes associated with this type of behavior. The mere presence of a female activates the hypothalamic-pituitary-gonadal complex, which manifests by increased plasma levels of luteinizing hormone and testosterone [2,6] and by movement towards the female [4,8]. After appearance of an estral female behind a transparent wall with small holes, blood testosterone level in the male considerably increases. The intensity of sexual stimulation depends on male genotype and environmental factors (season and stress) [2]. Previously we investigated (CBA and A/He) motivation and hormone components of sexual activation [4]. Appearance of a receptive female under conditions, when the male could see and smell it, but physical contact was impossible, led to an increase in blood testosterone level and characteristic behavioral changes (frequent excursions to the separating wall and longer time spent near this wall). Analysis of male behavior near the wall and dynamics of blood testosterone levels showed that behavioral changes preceded the increase in blood testosterone and did not result from increased level of this hormone.

We investigated the role of genotype in the intensity of behavioral component of sexual activation caused by the presence of a female (without direct physical contact between animals) and analyzed the correlation between the behavior and the reaction of the hypothalamic-pituitary-gonadal system in male mice.

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MATERIALS AND METHODS

Experiments were carried out on adult male mice (23-28 g) of 10 inbred strains (SWR, CBA, C3H/He, PT, BALB/c, C3HA/Y, C57Bl/6J, DBA/2, ICR, A/He) kept under standard vivarium conditions, 8 animals per groups. The tests were carried out in 28×14×10 cm cages with a separating plastic wall with small holes. Three days before the experiment the male was placed in one compartment for adaptation to new conditions and alleviation of the effect of social contacts. Five min before the experiment the metal cover of the cage was replaced with transparent plastic for the period of activation and adaptation of the males to new illumination conditions. Then a receptive female (BALB/c) was put into the empty compartment. Estrus was induced by injection of chorionic gonadotropin (10 U, prophasi, Serono) 24 h before experiment. The animals kept under similar conditions without females served as the controls. The groups consisted of 8-10 animals.

The time spent by males near the wall was evaluated [4]. Male behavior was studied for 30 min, the parameters were recorded every 5 min. After the experiment the animals were decapitated and blood testosterone levels serving as the hormonal indicator of the male sexual activity were measured by radioimmunoassay.

The results were processed by dispersion and regression analysis (ANOVA).

RESULTS

The time spent by male mice of different strains near the separating wall differed significantly ($F_{9.115}$=15.18, p<0.001; Fig. 1, *a*). This parameter clearly increased in response to the sex stimulus in males of all strains. It is noteworthy that characteristic behavior was observed during the very first 5 min, which manifested by movement towards the female, attempts to penetrate through the wall, and prolonged stay near the wall. The intensity of the behavioral component of sexual activation differed in different strains during the entire period of testing. The time spent near the wall was maximum in SWR mice and minimum (3-fold shorter) in A/He mice. Hence, behavioral reaction to the female is to a great extent genetically determined.

Paratypical comparison showed that the presence of a receptive female under condition excluding physical contact resulted in considerable activation of the pituitary-gonadal system in males ($F_{1.228}$=6.45, p<0.001; Fig. 1, *b*). Our results on mean testosterone levels for each genotype agree with published reports [2,5,7]. We observed a significant increase in blood testosterone level 30 min after the appearance of the female in males of 6 of 10 strains: a clear-cut increase in SWR, BALB/c, ICR, A/He, and CBA mice, insignificant increase in C3H/He and DBA/2 mice, and no activating effect was observed in C3HA/Y and C57Bl/6J mice. It is noteworthy that the behavioral component of sexual activation (time spent near the wall separating a receptive female) increased significantly in males of all studied strains, while hormone level sometimes remained unchanged. It can be hypothesized that the increase in blood testosterone level is weaker in males of these four strains and takes place at a different time.

Plasma testosterone level depends on the intensity of its secretion by testicular Leidig cells. We previously showed that the activating effect of the female

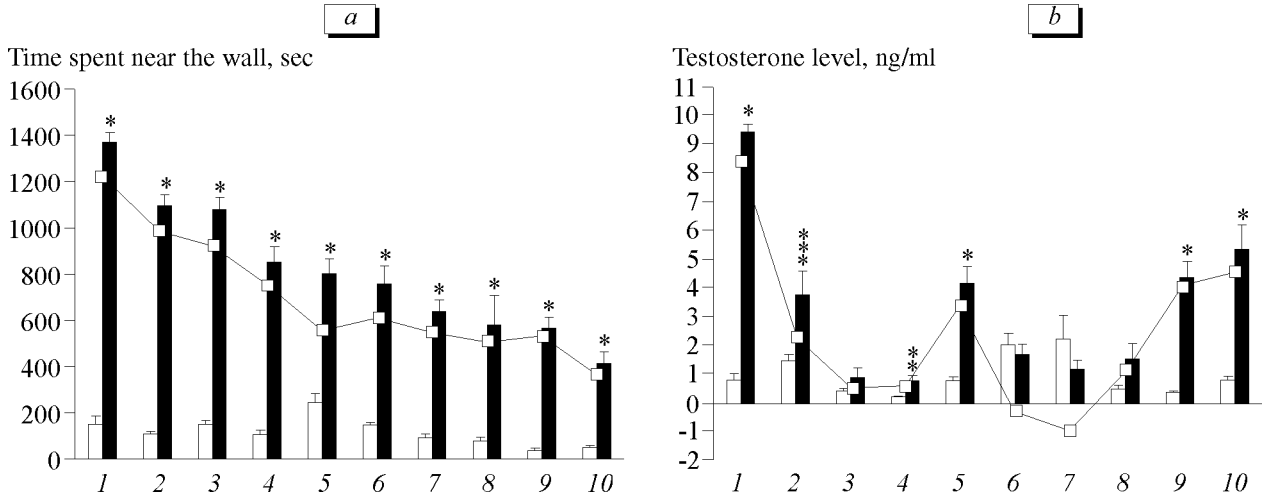

Fig. 1. Changes in motivation (*a*) and hormonal (*b*) components of sexual activation in male mice in response to 30-min presentation of a receptive female. Light bars: control; dark bars: sexual activation. Curves show activation-related increase in studied parameters. *1*) SWR; *2*) CBA; *3*) C3H/He; *4*) PT; *5*) BALB/c; *6*) C3HA/Y; *7*) C57Bl/6J; *8*) DBA/2; *9*) ICR; *10*) A/He. *p<0.001, **p<0.01; ***p<0.05 compared to the control.

on blood testosterone level is realized through the involvement of gonadoliberin receptors via luteinizing hormone, the basic regulator of testosterone biosynthesis [1]. Therefore, hereditary hormonal activity of Leidig cells can underlie the genetic differences in its plasma concentrations. Different genotypically determined reactions of Leidig cells to activating effect of chorionic gonadotropin and cAMP (analog of second intracellular mediator) in PT, CBA, A/He, and C57Bl/6J mice were previously demonstrated [3]. The highest reactivity of Leidig cells was observed in PT mice, the lowest in CBA and A/He mice. The same authors demonstrated genotypical differences in activity of steroidogenesis microsomal enzymes, the studied mouse strains ranking by their enzymatic activity in the same order, which can indicate a coordinated correlative pattern of genotypical variability in activities of steroidogenesis microsomal enzymes. The authors suggest a coordinating role of the factors regulating long-lasting changes in blood luteinizing hormone levels and the regulation of steroidogenesis enzyme gene expression.

Activation-related increase in the time spent near the wall and the rise of plasma testosterone level (difference between the parameters during sexual activation and in its absence) were calculated for each mouse strain. The maximum increase in both these parameters was observed in SWR mice, while in A/He mice the time spent near the wall increased insignificantly, while the increase in testosterone content was highly pronounced. The intensity of hormone response was higher in strains with medium initial hormone levels. The strains with initially low testosterone level responded to female appearance by minor changes in the hormone level (except ICR mice), while in some strains with initially high hormone level this parameter sometimes decreased (C3HA/Y and C57Bl/6J).

Regression analysis showed no relationships between activation-related prolongation of the stay near the wall and increase in testosterone level (b=0.004, $p>0.05$). A relationship between these two parameters of sexual activity of males (behavioral changes and hypothalamic-pituitary-testicular reaction) was observed during certain periods: between minutes 5-10 (b=12.5, $p<0.05$) and minutes 20-25 (b=12.5, $p<0.05$) after the appearance of a receptive female. However no genotypical relationship of this type between the two parameters of sexual activation of males was detected during the entire period of testing. We therefore suggest the presence of common regulatory (most likely neurotransmitter) mechanisms of behavioral and hormonal components of sexual activation; it seems that hereditary peculiarities of central mechanisms of regulation of these functions underlie the detected genetically determined variability of these components.

Comparative analysis of behavioral and hormonal components of sexual activation singled out mouse strains opposite by the studied signs. SWR and CBA genotypes demonstrated pronounced changes in the behavioral and hormonal components of sexual activation. High behavioral activity near the wall was associated with pronounced elevation of blood testosterone level in these animals. The opposite group of genotypes (DBA/2, C57Bl/6J) showed minor activation-related increase in behavior and no increase in hormone levels. The study of hereditary characteristics of the central mechanisms regulating sexual activity in animals of opposite groups seems to be very important.

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