Hormonal Reaction of the Testes to Chorionic Gonadotropin in CBA/Lac and PT Mice: Effects of the Dose and Time after Treatment

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Testosterone concentrations in the blood of CBA/Lac and PT mice and its content in the testes under normal conditions and during stimulation with chorionic gonadotropin were measured by enzyme immunoassay. Genetic differences in reactivity of the testes to chorionic gonadotropin were revealed: PT mice were characterized by more pronounced hormonal reaction compared to CBA/Lac mice. An optimal dose of chorionic gonadotropin (10 U) and time interval (120 min) for evaluation of potentialities of testicular hormonal function were determined.

Key Words: *testosterone; testes; inbreed mouse strains; chorionic gonadotropin*

Testosterone is a steroid hormone participating in the regulation of the formation of male phenotype and maintenance of reproductive function in male mammals. In male organism this hormone is primarily synthesized by Leydig cells of the testes and its production is controlled by luteinizing hormone (LH) of the pituitary [4]. The initial level of androgen activity of the testes under conditions of physiological "rest" usually does not determine the testicular endocrine function potential and does not reflect hormonal capacities of Leydig cells [2,6].

Chorionic gonadotropin (CG) is a functional analog of LH and its administration induces testosterone secretion in the testes.

We previously compared the production of testosterone by isolated Leydig cells at rest and during CG stimulation in mice of 6 inbreed strains: A/He, CBA/Lac, C57Bl/6J, DD, YT, and PT [2]. Under conditions of physiological rest, *in vitro* testosterone production by Leydig cells from PT and CBA/Lac male mice was similar, but stimulation with CG differentiated these strains attesting to higher steroidogenic potential of PT male mice [2]. It was interesting to determine the dose and time interval after CG administration *in vivo* optimal for detection of the differences between these strains.

Here we studied interstrain differences in testosterone level in the peripheral blood and testes under conditions of CG treatment *in vivo* in males of two inbreed strains CBA/Lac and PT opposite by reactivity of Leydig cells to CG.

MATERIALS AND METHODS

On the basis of experimental data obtained in Laboratory of Endocrinological Genetics, Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences, two inbreed mouse strains CBA/Lac and PT opposite by testosterone production by the testes under *in vitro* conditions were chosen for the experiments [1,2].

A total of 189 mature male CBA/Lac and PT mice at the age of 3 months were used. The mice were maintained under standard vivarium conditions (vivarium of Institute of Cytology and Genetics) at 24°C and regular illumination regimen 12/12 with water and food *ad libitum*. At the age of 1 month, the progeny was separated from mothers into standard plastic cages

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36×20×15 cm; sex-matched groups of 3-7 animals per cage were formed and maintained until the age of 90 days. Four days before the experiment, the animals were placed into individual cages to exclude the group effect.

Commercial human CG (Pregnyl, H.B. Organon) was dissolved in physiological saline to the required concentration. For evaluation of the dose of CG producing maximum hormonal response of the testes, the preparation was administered subcutaneously in doses of 5, 10, and 20 U in a volume of 100-200 µl 120 min before decapitation. For evaluation of the time interval after which the maximum hormonal response of the testes was observed, CG was administered subcutaneously in a dose of 10 U in a volume of 200 µl 20, 40, 60, 120, and 240 min before decapitation. The number of animals in each group varied from 10 to 15.

Preliminary experiments showed that administration of 200 µl physiological saline to males of both genotypes did not affect testosterone concentration in the blood and its content in the testes, therefore intact animals (without saline injection) were used as the control.

The males were decapitated from 13.00 to 14.00, the peripheral blood was collected and the testes were removed. Peripheral blood was centrifuged at 4°C and 3000 rpm for 20 min, the serum was stored at -40°C. The testes were homogenized in phosphate buffer, centrifuged, and the supernatant was stored at -40°C.

Testosterone concentration in the serum and its content in homogenates of the testes were measured by EIA using SteroidIFA-testosterone-01 kits (Alkor Bio) according to manufacturer's protocol. Calibration curve for calculation of testosterone content in the testes was plotted using testosterone standard diluted with phosphate buffer, and the curve for calculation of blood hormone concentration was constructed using the standard diluted with steroid-free mouse serum.

The data were processed statistically using two-way ANOVA. The sample mean and error of the mean were calculated. The differences were significant at $p<0.05$. For group comparison, Duncan multiple comparison test was used. Pearson correlation coefficient was used for evaluation of the correlation between the level of testosterone in the peripheral blood and testes for each genotype.

RESULTS

Two-way ANOVA (the main factors were CG dose and mouse genotype) showed that serum level of testosterone depended on both the dose of CG ($F(3,99)=54.05$, $p<0.001$) and animal genotype ($F(1,99)=119.94$, $p<0.001$). A significant interaction of the main factors was observed ($F(3,99)=10.92$, $p<0.001$). In males of both mouse strains, blood level of testosterone attained the maximum after injection of 5 U CG ($p<0.001$) and did not further increase with increasing the dose of the preparation (Fig. 1, *a*).

Two-way ANOVA showed that the level of testosterone in the testes was affected by both the dose of CG ($F(3,98)=52.27$, $p<0.001$) and animal genotype ($F(1,98)=26.15$, $p<0.001$). A significant interaction of the main factors was observed ($F(3,98)=6.26$, $p<0.001$). In PT males, testosterone content in the testes attained maximum values after injection of CG in a dose of 20 U and differed significantly from the basal level ($p<0.001$). In CBA/Lac males, testosterone content in the testes attained maximum after injection

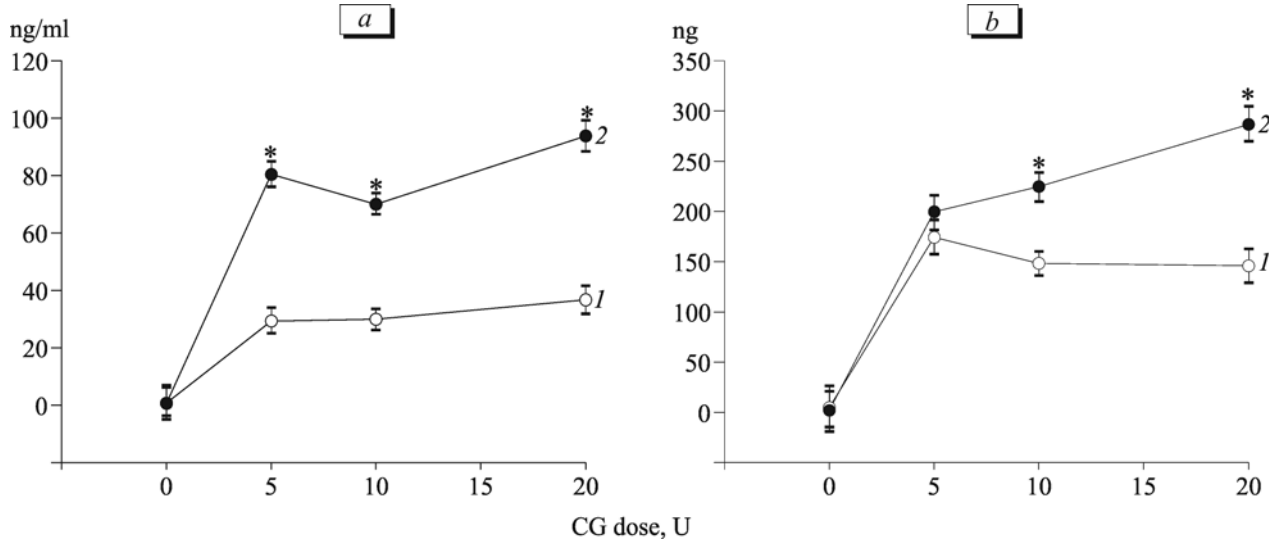

Fig. 1. Concentration of testosterone in the blood (*a*) and its content in both testes (*b*) in CBA/Lac (*1*) and PT mice (*2*) after subcutaneous injection of CG. Here and on Fig. 2: *$p<0.05$ compared to CBA/Lac.

of 5 U CG (p<0.001) and then did not increase with increasing the dose of the preparation (Fig. 1, *b*).

Two-way ANOVA (the main factors were time after CG injection and mouse genotype) showed that serum level of testosterone depended on both time after CG injection ($F(5,68)=50.45$, p<0.001) and animal genotype ($F(1,68)=10.59$, p<0.001). A significant interaction of the main factors was observed ($F(5,68)=4.52$, p<0.002). In males of both mouse strains, blood level of testosterone attained the maximum 120 min after CG injection (p<0.001) and did not further increase with increasing the time interval (Fig. 2, *a*).

Similar data were obtained for testosterone content in the testes. It was found that the level of testosterone in the testes was affected by both time after CG injection ($F(5,68)=49.77$, p<0.001) and animal genotype ($F(1,68)=17.17$, p<0.001). A significant interaction of the main factors was observed ($F(5,68)=4.07$, p<0.003). In males of both mouse strains, testosterone content in the testes attained maximum 120 min after CG injection (p<0.001) and did not further increase with increasing the time interval (Fig. 2, *b*).

Thus, interstrain differences by blood level of testosterone were clearly seen even after injection of CG in a dose 5 U and persisted after increasing the dose. However, interstrain differences by the content of testosterone in the testes were observed after injection of 10 U CG and persisted after increasing the dose (Fig. 1). Thus, 10 U is the optimal dose of CG differentiating the genotypes. Evaluation of the temporal dynamics of the testicular response to CG injection showed that 120 min is the optimal interval for detection of interstrain differences.

We revealed a significant (p<0.05) correlation between blood level of testosterone in the blood and its content in the testes after injection of CG in male PT (r=0.95) and CBA/Lac mice (r=0.89).

Evaluation of the hormonal reaction of the testes to injection of CG showed that PT males were more reactive to CG than CBA/Lac males. These conclusions agree with the previous data obtained on suspension of Leydig cells of the testes [2].

Genetic differences by blood level of testosterone and its content in the testes can be explained by genetically determined peculiarities of biosynthesis, metabolism, and excretion of the hormone. For instance, interspecies differences of the basal blood level of testosterone in male mice can be determined by different functional activity of the pituitary [4]. On the other hand, injection of the same dose of CG *in vivo* (50 ng) to male mice of 6 inbreed strains (A/He, CBA/Lac, C57Bl/6J, DD, YT, and PT) stimulated the appearance of interstrain differences by blood level of testosterone [7]. Hence, potentialities of hormonal functions of the testes can be determined by the power of the "steroidogenic machinery" of Leydig cells.

We can hypothesize that the interstrain differences observed by us are due to genetically-determined differences in activity of steroidogenesis enzymes. This assumption is confirmed by the data on interstrain differences in the key enzymes of steroidogenesis in Leydig cells [1].

On the other hand, previous studies demonstrated the leading role of LH receptors on membrane of Leydig cells in the formation of hormonal response of the testes [8]. Thus, the revealed interstrain differences in the blood level of testosterone and its content in the testes can be determined by different number and/or functional activity of receptors to LH. Moreover, the formation of the endocrine response of Leydig cells is

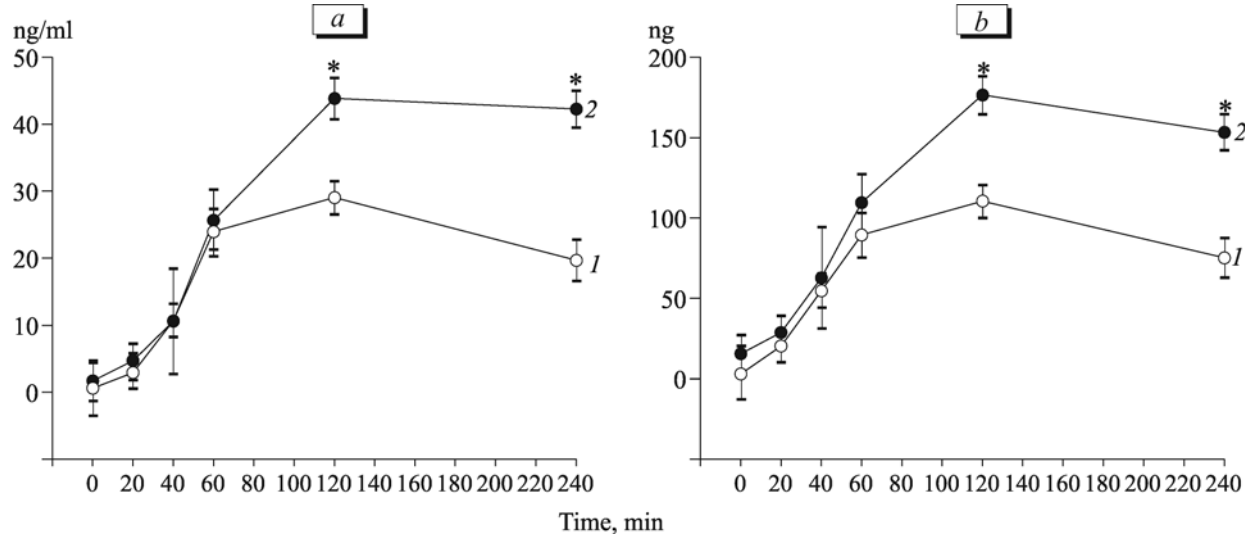

Fig. 2. Concentration of testosterone in the blood (*a*) and its content in both testes (*b*) in CBA/Lac (*1*) and PT mice (*2*) at different terms after subcutaneous injection of CG in a dose of 10 U.

modulated by insulin-like growth factor-1 synthesized in Sertoli cells and promoting expression of LH receptors on Leydig cells [3].

Testosterone from Leydig cells diffuses into the seminiferous tubules and modulates the process of spermatogenesis [5]. Thus, we can hypothesize that interstrain differences in the testicular reaction to steroidogenesis stimulator can indirectly induce changes in spermatogenesis determining lower parameters of spermatogenesis in CBA/Lac mice compared to PT mice.

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