EXPERIMENTAL GENETICS

Genetic Basis of Adenylatecyclase Regulation of Testosterone Production of Leydig's Cells in Laboratory Mice

A. V. Osadchuk and K. V. Svechnikov

UDC 612.616.3.018:577.175.624].08

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 118, № 8, pp. 177-179, August, 1994
Original article submitted December 16, 1993

The function of Leydig's cells during stimulation of various components of the adenylatecyclase system was studied in inbred mice of various lines. Mice of different lines were found to differ markedly in their production of testosterone during stimulation of a crude Leydig's cell suspension with ascending concentrations of chorionic gonadotropin, cholera toxin, forskolin, and dibutyryl-cAMP. The ranking of the lines according to the maximal production of testosterone was virtually the same during exposure to steroidogenesis activators.

Key Words: *Leydig's cells; testosterone production; genetic differences; inbred lines of mice; chorionic gonadotropin; cholera toxin; forskolin; dibutyryl-cAMP*

The genetic control of gonadal hormonal regulation is a current topic in the biology of animal breeding. Inbred lines of mice, which are relatively homogeneous genetically, are a convenient object for such studies. Previously we found marked hereditarily determined differences between lines in plasma testosterone levels both in health at rest and during some types of zoosocial interactions [1,3]. Further studies carried out on 6 lines of mice with different blood testosterone levels revealed an appreciable genetic polymorphism in the production of testosterone by decapsulated testicles *in vitro* under the effect of chorionic gonadotropin and cAMP [2]. All these facts pointed to marked genetic differences in the regulation of testosterone biosynthesis by Leydig's cells of inbred mice of different strains. However, the mechanism of genetic control determining these differences is still little known, and only a few reports on this subject are available [9].

We carried out a comparative genetic analysis of testosterone production by Leydig's cells during stimulation of various components of the adenylate-cyclase (AC) system in 6 inbred strains of mice with different plasma levels of testosterone [1,3], *in vitro* testosterone production by the testicles [2], and the reproductive output and level of zoosocial dominance [4].

Department of the Genetic Basis of Neuroendocrine Regulation, Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk. (Presented by L. E. Panin, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Experiments were carried out with adult male mice of six inbred lines: CBA/Lac, A/He, C57Bl/6J, DD, YT, and PT. Leydig's cells were isolated from the testicles by the mechanical method [10], after which they were passed through a nylon filter and centrifuged at 150 g for 5 min. The sediment was resuspended in 2 ml Eagle's medium presaturated with

0007-4888/94/0008-0871$12.50 ©1995 Plenum Publishing Corporation

carbogen (95% O_2 and 5% CO_2) and containing 0.1% bovine serum albumin. Leydig's cells suspended in Eagle's medium with phenol red (10 mg/liter) were counted in a Goryaev chamber using a phase-contrast microscope, as described previously [8]. In our experiment, using the indicated method of Leydig cell isolation, these cells constituted about 40 to 50% of the total amount of cells. All subsequent experiments were carried out with a crude suspension of Leydig's cells. Such an approach was used previously to assess the reactivity of Leydig's cells during stimulation with chorionic gonadotropin [11].

Leydig's cells, 1.25×10^5 cells/ml (5×10^4 cells per sample) were incubated in Eagle's medium with the following agents: chorionic gonadotropin (Calbiochem) 0.2 to 150 mU; cholera toxin (Sigma) 0.06 to 45 ng/ml; dibutyryl-cAMP (Calbiochem) 1.1 to 800 μM; and forskolin (Calbiochem) 0.04 to 32 μM. The samples were incubated in a bath with a shaker for 3 h at 34°C in a medium with 95% O_2 and 5% CO_2. After incubation the samples were placed in an ice bath with 0.1 ml of 3 M $HClO_4$ added to destroy the cells and 0.35 ml of 1 M KOH to neutralize the incubated mixture. After 10 min centrifugation at 150 g the samples were poured into tubes and stored at -20°C prior to measurement of the testosterone level in them. The testosterone content was radioimmunoassayed in the samples using a highly specific antiserum to testosterone.

The results were statistically processed by two-factor analysis of variance with due consideration for the nonuniformity of the variances in the experimental groups [5].

RESULTS

Figure 1 shows that chorionic gonadotropin greatly boosted testosterone production in Leydig's cells of mice of all strains ($F_{7.19}=38.8$, $p<0.001$). Another factor appreciably influencing this parameter is the genotype of the line ($F_{5.19}=34.1$, $p<0.001$). Of special interest is the highly reliable interaction between the studied factors - genotype and stimulant dose - which is indicative of a different genotype-dependent reaction of Leydig's cells to chorionic gonadotropin ($F_{35.19}=4.0$, $p<0.005$). PT mice were the most reactive to the hormone. The highest increment of testosterone production, surpassing the control value 26-fold, was observed in these animals. On the other hand, another strain of mice, CBA/Lac, was characterized by the lowest testosterone production in response to stimulation with chorionic gonadotropin. The maximal increase of the hormone in these animals surpassed the baseline level only 6-fold. The other four strains of mice were intermediate in their ability to produce testosterone during stimulation with chorionic gonadotropin. Analysis of the causes of such pronounced differences among the lines led us to hypothesize that there are genetically determined structural changes in AC proteins which might influence the enzyme activity and, hence, the production of cAMP and testosterone. To check this hypothesis we used two activators of this enzyme, cholera toxin and forskolin.

Cholera toxin is known to have a stimulating effect on AC by ADP ribosylation of G_s-protein, this resulting in constant activation of the enzyme, despite the absence of the hormone [12]. Our experiments with cholera toxin showed that the effects of the studied factors (genotype and stimulant dose) manifest themselves similarly as in experiments with chorionic gonadotropin. First, the toxin induced a marked stimulation of testosterone production ($F_{7.15}=16.1$, $p<0.001$). Second, the observed differences among the lines in terms of this parameter were pronounced ($F_{5.15}=33.1$, $p<0.001$). And, third, a reliable genotype-dependent type of reaction to cholera toxin was detected ($F_{35.15}=3.1$, $p<0.025$). Similarly as with chorionic gonadotropin, the maximal production of testosterone was observed in PT mice. The peak of testosterone production in these mice surpassed the control values 22 times (Fig. 1). In contrast to PT mice, in CBA/Lac males, just as in experiments with chorionic gonadotropin, the capacity of cells to generate testosterone in response to stimulation with cholera toxin was the lowest. Hormone production increased only five-fold in comparison with the control. Mice of the remaining strains ranked in the same order by their capacity to generate testosterone after stimulation with cholera toxin, as in experiments with chorionic gonadotropin. These results suggest that hereditary differences in the production of testosterone in the tested strains of mice are not related to the activity of AC G_s-protein.

Another AC activator, forskolin, mediated its stimulating effect on steroidogenesis through activation of an AC catalytic subunit [7]. This preparation also caused an increase of testosterone production by murine Leydig's cells ($F_{7.26}=8.3$, $p<0.001$), but its effect on steroidogenesis was less pronounced than that of chorionic gonadotropin and cholera toxin. Similar results were obtained by other authorities [6], who came to the conclusion that forskolin is a far less effective activator of steroidogenesis than gonadotropins. Clear-cut hereditary differences in testosterone production under the effect of forskolin were revealed ($F_{5.26}=14.4$, $p<0.001$), but we did not find any interaction between the exam-

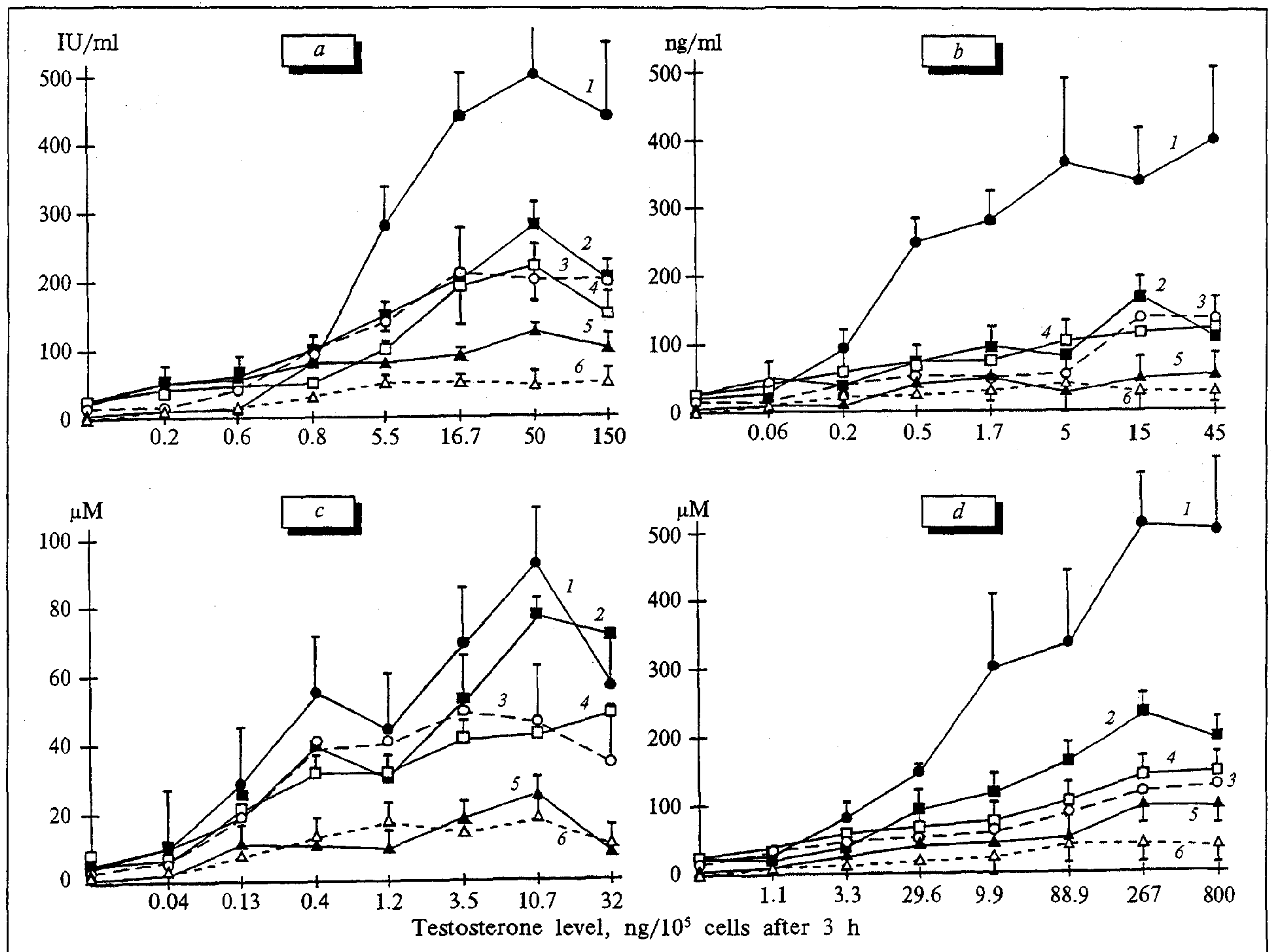

Fig. 1. Effect of chorionic gonadotropin (*a*), cholera toxin (*b*), forskolin (*c*), and cAMP (*d*) on testosterone production by Leydig's cells of inbred mice. Abscissa: concentrations of preparations. Inbred strains of mice: *1*) PT; *2*) YT; *3*) DD; *4*) C57Bl/6J; *5*) A/He; *6*) CBA/Lac.

ined factors. At the same time, forskolin did not alter the previously established ranking of each murine strain according to its capacity to produce testosterone. For example, in PT and CBA/Lac mice, showing the most contrasting capacities to synthesize testosterone, the maximal increase of its production after stimulation with forskolin exceeded the control value by 5 and 2 times, respectively (Fig. 1). Hence, differences in testosterone production among the tested murine lines did not depend on the activity of the AC catalytic subunit.

The next series of experiments examined the direct stimulating effect on testosterone production of a secondary intermediate, dibutyryl-cAMP. This cyclic nucleotide caused a pronounced activation of testosterone generation by Leydig's cells of mice of all strains ($F_{7.16}$=24.4, p<0.001). As with other steroidogenesis stimulants, we revealed highly reliable differences in testosterone production by murine cells of different lines under the effect of cAMP ($F_{5.16}$=31.7, p<0.001) and established a genotype-dependent pattern of reactivity to this cyclic nucleotide ($F_{35.16}$=3.2, p<0.01). Specifically, the maximal production of testosterone by Leydig's cells of PT mice was 27 times higher than the baseline value (Fig. 1), whereas Leydig's cells of CBA/Lac mice, the least active strain in this respect, produced only 6 times higher amounts of the hormone in comparison with the control. As for the other 4 murine strains, they ranked in virtually the same order by the capacity to produce the androgen in response to stimulation with cAMP as in experiments with other agents.

Hence, we revealed pronounced hereditary differences in testosterone production by Leydig's cells of different murine strains during stimulation of various components of the AC system: of the receptor component with chorionic gonadotropin, of AC G_s-protein with cholera toxin, of the AC catalytic subunit with forskolin, and directly with a

secondary intermediate analog dibutyryl-cAMP. It is noteworthy that the ranking of strains by the maximal production of testosterone was virtually the same under the effect of the said steroidogenesis activators. The differences in testosterone production were maximal (10-fold) between the cells of PT and CBA/Lac mice. The persistence of genetic differences in the production of testosterone after stimulation of various components of the AC system allows us to speculate that the key (causative) hereditary factors underlying the revealed genetic polymorphism of Leydig cell hormonal activity should be sought in the stages of steroid generation distal from cAMP production.

REFERENCES

1. A. V. Osadchuk, in: *Ontogenetic and Genetic Evolutionary Aspects of Neuroendocrine Regulation of Stress* [in Russian], Novosibirsk (1990), pp. 160-170.
2. A. V. Osadchuk, T. V. Kutuzova, and E. V. Naumenko, in: *All-Union Congress of Endocrinologists* [in Russian], Tashkent (1989), p. 77.
3. A. V. Osadchuk and E. V. Naumenko, *Dokl. Akad. Nauk SSSR*, **258**, № 3, 746-749 (1981).
4. A. V. Osadchuk and E. V. Naumenko, *Ibid.*, **291**, № 1, 253-256 (1986).
5. G. Scheffe, *The Analysis of Variance*, Wiley, New York (1957).
6. M. L. Dufau, A. Khanum, C. A. Winters, and Ch.-H. Tsai-Morris, *J. Steroid Biochem.*, **27**, 343 (1987).
7. A. Lefevre, C. Finaz, M. C. Berthelon, and I. M. Saez, *Molec. Cell. Endocr.*, **40**, 107-114 (1985).
8. M. Schumacher, G. Schafer, A. F. Holstein, and H. Hilz, *FEBS Lett.*, **91**, № 2, 333-338 (1978).
9. J. R. D. Stalvey and A. H. Paine, *Endocrinology*, **112**, 1696-1701 (1983).
10. S. Stoklosowa, *Acta Biol. Acad. Sci. Hung.*, **33**, № 4, 367-379 (1982).
11. M.-P. Van Damme, D. M. Robertson, and E. Diczfalusy, *Acta Endocrin.*, **77**, 655-671 (1974).
12. S. Van Heyningen, *Biosci. Rep.*, **2**, 135 (1982).