GENETICS

Effect of Genotype on Hormonal Activity of Leydig's Cells in Inbred Mice with Stress

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Acute stress depresses the endocrine function of Leydig's cell in PT and CBA/Lac mice, whereas in A/Sn and BALB/c mice blood testosterone level and the function of Leydig's cells are virtually intact in stress. This difference can be due to genetic differences in the reactions of the hypothalamo-pituitary-gonadal and hypothalamo-pituitary-adrenal systems.

Key Words: *testosterone; Leydig's cells; acute stress; inbred mice*

Study of the effects of stress factors on the gonadal endocrine function is an important problem of biology of animal reproduction. Stress can decrease and increase for a short time the level of testosterone (TS) in the plasma of mice and rats [1,6,7]. These differences can be caused by genetically determined reactions of Leydig's cells (LC) to stress. The fact that the direction and degree of gonadal reaction to stress depend on the animal genotype [1] confirm this hypothesis. However, hereditary mechanisms regulating the LC reaction to stress are still unknown. Genetically homogenous inbred strains of mice are a convenient tool for the investigation of this problem.

Mechanisms regulating androgen production under conditions of emotional stress are studied in 4 inbred mouse strains contrast by the blood TS level [2] and TS production by LC [3] *in vivo* and *in vitro* in isolated LC cells.

MATERIALS AND METHODS

Experiments were carried out on adult male mice of 4 inbred strains: CBA/Lac, PT, BALB/c, and A/Sn. Emotional stress was produced by limiting the mobility of animals which were put in small cylindrical metal cages 3 cm in diameter for 1 h. After stress, the animals were decapitated, blood was collected and centrifuged at 3000 rpm for 15 min. Plasma was stored at -20°C until TS measurements. Intact mice were controls.

Leydig's cells were isolated from the gonads by the mechanical method [14]. Crude suspension of LC was purified on a Percoll density gradient as described previously [5].

The cells (1.25×10^5 cells/ml, or 5×10^4 cells per sample) were incubated in Eagle's medium with chorionic gonadotropin (Calbiochem) 50 mU; dibutyryl-cAMP (Calbiochem) 250 μM; pregnenolone, progesterone, and androstenedione (Sigma) 10 μM. Incubation with pregnenolone, progesterone, and androstenedione was performed in order to measure the activities of enzymes participating in TS production through the Δ^4 pathway: 3β-hydroxysteroid dehydrogenase, cytochrome $P\text{-}450_{17\alpha}$, and ketosteroid reductase. Incubation was carried out in a bath with a shaker for 3 h at 34°C in medium with 95% O_2 and 5% CO_2. After incubation, the samples were put in an ice bath and then centrifuged at 1500*g* for 10 min. Supernatant was poured in tubes and stored at -20°C. Testosterone in the tubes was radioimmunoassayed with highly specific antiserum. Cross reaction of antiserum with structurally related steroids was

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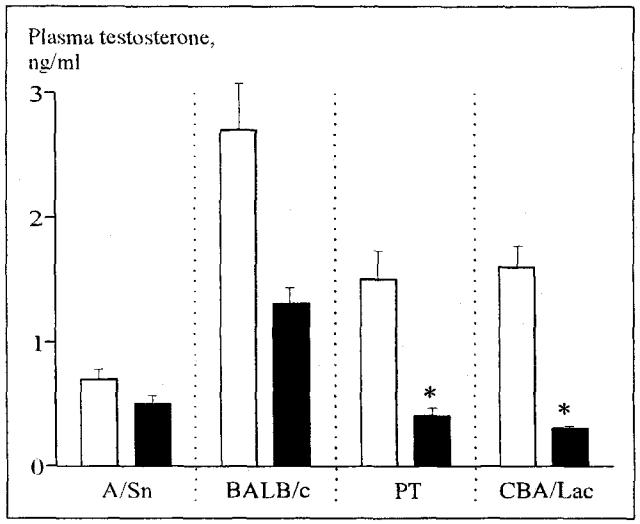

Fig. 1. Changes in plasma testosterone level in inbred mouse strains exposed to emotional stress. Here and in Fig. 2: light bars — intact animals; dark bars — stress.

0.03% for pregnenolone, 0.06% for progesterone, and 5.6% for androstenedione, which did not impede TS measurements in incubated samples.

The results were processed using Student's *t* test.

RESULTS

Figure 1 shows that emotional stress caused by limitation of mobility markedly decreased the level of TS in the blood of examined mouse strains. Stress-induced decrease in TS was the greatest in CBA/Lac and PT mice: 3.5 and 5.6 times, respectively, in contrast to a tendency in BALB/c mice. In A/Sn mice, TS level was virtually not changed after stress exposure. These data are in line with our previous results [1] and reports of other scientists [9,10] showing that acute stress causes a drop in plasma TS. Change in blood TS level in mice of the studied strains indicated a probable effect of stress on the endocrine function of LC, the main site of TS production in the organism [12]. Functional activity of LC was studied by stimulating various components of the hormone production: the receptor component was stimulated by chorionic gonadotropin, adenylate cyclase by dibutyryl-cAMP and the TS precursors pregnenolone, progesterone, and androstenedione. Analysis of LC reaction to *in vitro* stimulation under conditions of emotional stress demonstrated a reaction similar to that observed *in vivo* (Fig. 2). Stress appreciably decreased the reactivity of LC to chorionic gonadotropin and dibutyryl-cAMP in PT mice. In addition, we should like to note a tendency to a decrease in TS production by LC incubated with the hormone precursor. The total effect of all these processes apparently causes a decrease in blood TS level in PT mice exposed to stress. The blood TS of CBA/Lac mice reacted to stress similarly. However, the reactions of LC to stimulation under conditions of emotional stress were variously directed in this mouse strain. The production of TS by LC had a tendency to decrease under the effect of dibutyryl-cAMP, pregnenolone, and progesterone and to increase after incubation with chorionic gonadotropin and androstenedione in comparison with intact animals. However, the result was a decrease in androgen production by LC of stressed CBA/Lac mice. In contrast to PT and CBA/Lac mice, the response of LC to emotional stress in A/Sn and BALB/c mice was weak. Only in A/Sn mice intensification of pregnenolone metabolism to TS was observed, probably due to activation of 3β-hydroxysteroid dehydrogenase. Stimulation of the other components of TS production revealed no notable changes in the hormone production by LC in mice of the studied strains exposed to stress. There was a tendency toward intensification of androstenedione metabolism to TS in both strains. The decrease in blood TS level in emotionally stressed A/Sn and BALB/c mice can be due to hemodynamic causes, such as decreased blood flow in the gonads induced by an increase in plasma catecholamines. Moreover, we revealed hereditary differences between the strains in the production of TS by LC stimulated with different activators of steroidogenesis in intact animals. It is noteworthy that the strains ranked by the maximum production of TS in the same order as was determined previously [3,4].

Thus, we detected differences in the reactivity of LC to acute emotional stress in different strains of mice. Stress inhibits the endocrine function of LC cells in PT and CBA/Lac mice but virtually does not affect it in A/Sn and BALB/c mice. The causes of these differences are not clear because of the intricate mechanism of stress effect on sexual function. Stress hormones can influence gonadal endocrine function at all three levels of the hypothalamo-pituitary-gonadal complex: in the hypothalamus they inhibit the production of gonadotropin releasing factor, in the pituitary decrease the production of luteinizing hormone (LH), and in the gonads change the reactivity of LC to gonadotropins [11]. Thus, it is probable that the suppressive effect of stress on the production of TS by LC is caused primarily by decreased production of LH. A drop of this hormone in the blood of mice exposed to emotional stress was reported previously [6]. But LH is not the only modulator of LC function in stress. Other hormones inhibiting the gonadal function are corticosteroids. These hormones can inhibit the production of TS at stages of cholesterol lateral chain cleavage and progesterone metabolism to androstenedione [8,9] and decrease gonadal sen-

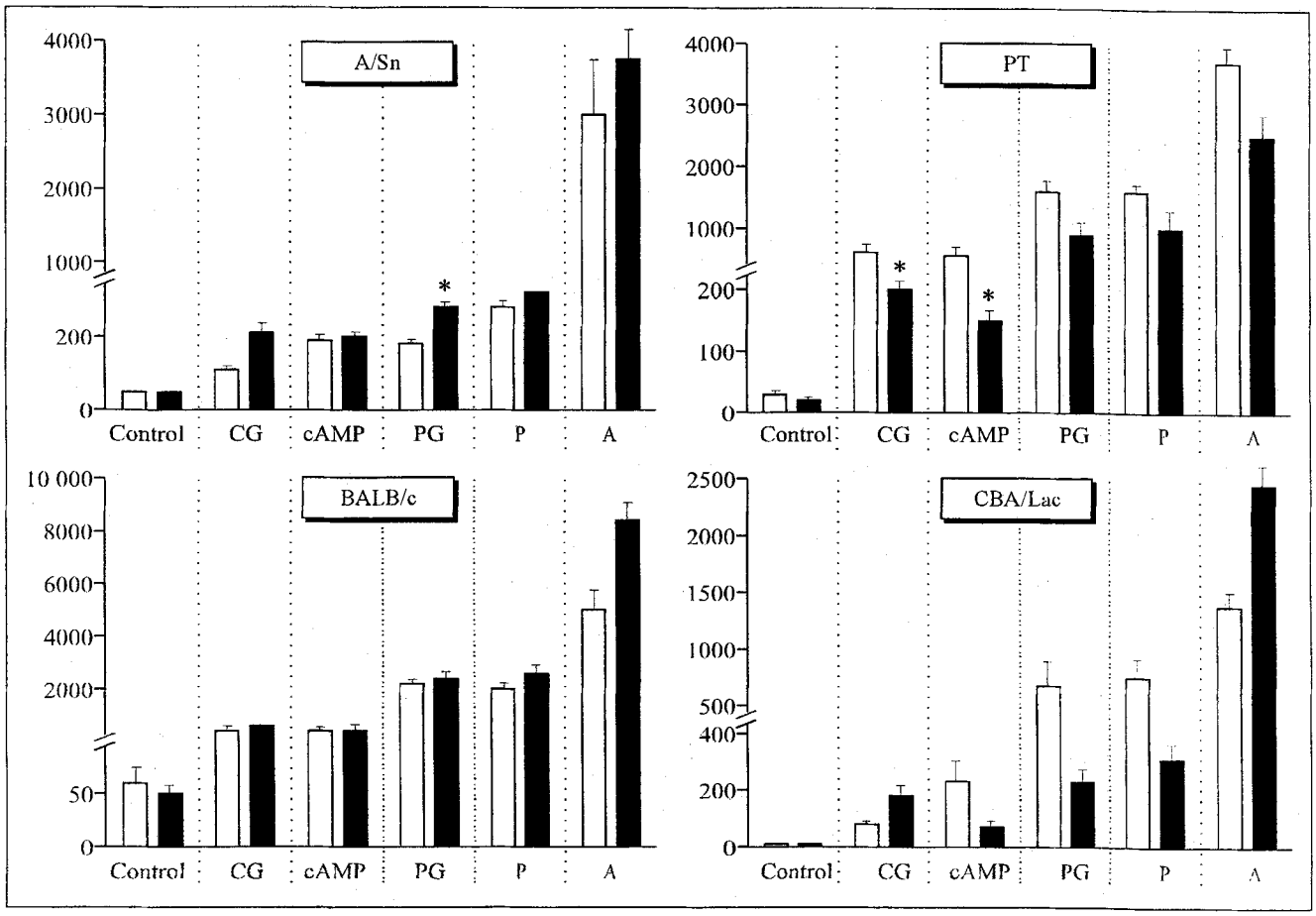

Fig. 2. Effect of emotional stress on testosterone production by Leydig's cells in inbred mice. Ordinate: testosterone level, ng/10^5 cell in 3 h; abscissa: CG) chorionic gonadotropin; PG) pregnenolone; P) progesterone; A) androstenedione.

sitivity to gonadotropins [13]. Corticosteroids can play the main role in the decrease of the endocrine function of LC in PT mice, because in these animals the increment of these hormones' level in emotional stress is the greatest [2].

Thus, the gonadal endocrine function of different mouse strains responds differently to acute emotional stress. PT and CBA/Lac mice are highly sensitive to the depressive effect of acute stress, whereas A/Sn and BALB/c mice virtually do not react to emotional strain. These differences can be due to genetically determined reactions of the hypothalamo-pituitary-gonadal complex to stress and higher sensitivity of the gonads to stress hormones in PT and CBA/Lac mice.

REFERENCES

1. A. V. Osadchuk, in: *Ontogenetic and Genetic Evolutional Aspects of Neuroendocrine Regulation of Stress* [in Russian], Novosibirsk (1990), pp. 160-170.
2. A. V. Osadchuk and E. V. Naumenko, *Dokl. Akad. Nauk SSSR*, **258**, No. 3, 746-749 (1981).
3. A. V. Osadchuk and K. V. Svechnikov, *Byull. Eksp. Biol. Med.*, **118**, No. 8, 177-180 (1994).
4. A. V. Osadchuk and K. V. Svechnikov, *Dokl. Ross. Akad. Nauk*, **343**, No. 2, 281-283 (1995).
5. K. V. Svechnikov, T. V. Busygina, and A. V. Osadchuk, *Vopr. Med. Khimii*, No. 4, 14-17 (1994).
6. F. N. Bronson, *Physiol. Behav.*, **10**, 947-951 (1973).
7. J. S. Euker, J. Meites, and G. D. Riegele, *Endocrinology*, **96**, 85-92 (1975).
8. D. B. Hales and A. H. Payne, *Ibid.*, **124**, 2099-2104 (1989).
9. T. E. Orr, M. F. Taylor, A. K. Bhattachargya, and D. C. Collins, *J. Androl.*, **15**, 302-308 (1994).
10. J. A. Ramaley, in: *Environmental Factors in Mammal Reproduction*, London (1981), pp. 127-137.
11. C. Rivier and S. Rivest, *Biol. Reprod.*, **45**, 523-532 (1991).
12. F. F. G. Rommerts, G. H. Barker, R. Molenaar, and N. J. Van der Molen, *Biochem. Endocrin.*, **1**, 293-313 (1982).
13. J. M. Saiz, A. M. Morera, F. Haour, and D. Evain, *Endocrinology*, **101**, No. 4, 1256-1263 (1977).
14. S. Stoclosowa, *Acta Biol. Acad. Sci. Hung.*, **33**, No. 4, 367-379 (1982).