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Gamma-Aminobutyric Acid Controls the Mouse Hypothalamic-Pituitary-Testicular Response to the Presence of Female

E. V. NAUMENKO AND L. I. SEROVA

Institute of Cytology and Genetics, Siberian Branch of the Academy of Sciences, 630090, Novosibirsk, USSR

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NAUMENKO, E. V. AND L. I. SEROVA. *Gamma-aminobutyric acid controls the mouse hypothalamic-pituitary-testicular response to the presence of female.* PHARMACOL BIOCHEM BEHAV **40**(2) 287–290, 1991.—The role of gamma-aminobutyric acid (GABA) in the control of plasma testosterone was studied on male mice of inbred strains (CBA/Lac, A/He and BALB/c) exposed to a sexually receptive female in the same cage but separated by a partition. Within 40 minutes, testosterone levels in plasma increased 1.5–3.5 times depending upon the mouse genotype. This process could be completely blocked if GABA accumulation was induced by pretreatment with the GABA transaminase inhibitor, aminooxyacetic acid (AOAA), or by emotional stress induced by 40 min of restraint. Neither bicuculline-induced blockade of GABA receptors nor a decrease of GABA concentration induced by prior administration of thiosemicarbazide (an inhibitor of glutamate decarboxylase), affected the increase of plasma testosterone that occurred in response to presentation of a receptive female. However, at sexual arousal, the bicuculline blockade of GABA receptors significantly reduced the inhibitory effects of both AOAA administration and emotional stress on plasma testosterone levels. We conclude that the inhibitory effect of emotional stress on female-induced activation of testicular endocrine function is mediated, at least in part, via activation of bicuculline-sensitive receptors.

Plasma testosterone Emotional stress GABAergic mechanism Sexual arousal Aminooxyacetic acid Thiosemicarbazide Bicuculline

GAMMA-AMINOBUTYRIC ACID (GABA), an inhibitory neurotransmitter within the central nervous system, participates in the neuronal regulation of the hypothalamic-pituitary-testicular complex (10–12, 24, 30, 32). GABAergic transmission also controls copulatory and ejaculatory behaviour (1, 2, 4, 14, 15, 22). The role of GABA in regulating sexual motivation is, as yet, imperfectly understood. Nevertheless, it was shown that GABAergic neurotransmission within the preoptic-hypothalamic region is involved in an inhibitory process underlying sexual motivation. This was demonstrated by mating tests performed on castrated male rats presented with a stimulus female after the injection of bicuculline (a GABA receptor antagonist) into the medial preoptic-anterior hypothalamic area of males (15). It is known, however, that sexual arousal appears without the need for direct contact between a male and a sexually receptive female and is due mainly to activation of olfactory receptors specific to rodents. The presence of a receptive female in the same cage as a male, but separated by a wire partition, leads to an increase of blood testosterone levels in male mice (5, 28, 31) and guinea pigs (18). This phenomenon depends upon LH-RH activation of LH-RH receptors since their blockade, or hypophysectomy, completely abolishes the stimulatory effect of a female presence on the plasma levels of testosterone (3). To our knowledge, the neurochemical processes which participate in the control of the testosterone response of male mice to the presence of a receptive female have not been studied. Our recent results (27) enable us to conclude that, under certain conditions, the noradrenergic system may participate in regulation of testicular endocrine function.

The goal of the present study was to elucidate the role of GABAergic processes in control of blood testosterone levels in male mice in response to presentation of a sexually receptive female. The results have already been partially published (29).

METHOD

Animals

Three-month-old inbred strains of mice (CBA/Lac, A/He, BALB/c) were used. The animals were born and bred under natural illumination at the vivarium of the Institute of Cytology and Genetics of the USSR Academy of Sciences. The animals were given food and water ad lib.

Procedures

Five days before experimentation, mice were placed individually into cages ($28 \times 14 \times 10$ cm) which were divided into two equal parts by wire partitions. Every day the animals were subjected to 5 min of handling to reduce their responsiveness to subsequent drug administration. After five days, each male was subjected to a 40-min presentation with a stimulus female (strain BALB/c, beyond the partition inside the cage) brought into estrus by subcutaneous administration of 0.1% estradiol benzoate and 1% progesterone in 0.06 ml of olive oil made 54 and 6

hours before testing, respectively.

Emotional stress was performed by restricting the mobility of mice inside the cage. For this purpose, at the moment when a female was placed into a free compartment of the cage, the male was put into a narrow net cylinder (dia. = 2.5 cm, l = 8 cm) and returned to the compartment where it was kept earlier. In other experiments, emotional stress was achieved as follows: six sexually receptive females were placed for 40 min into the populated cages (74 × 46 × 17 cm), which, previously, had been occupied by groups of 6 males for 7 days.

Forty minutes after presentation of a sexually receptive female, or a corresponding period of time for control experiments, the males were decapitated (1600 h). Trunk blood was collected in heparinized tubes, centrifuged and the plasma obtained was stored at −40°C until it was analysed. Testosterone in plasma was measured by radioimmunoassay using a highly specific antiserum and ^{3}H-testosterone (Amersham). Corticosterone was determined by the competitive protein binding method (25).

Drugs

To elevate the GABA levels in brain (35), we used an inhibitor of GABA-transaminase, aminooxyacetic acid (AOAA, Sigma), in doses of 15 and 20 mg/kg. To decrease the GABA levels (11) an inhibitor of glutamate-decarboxylase, thiosemicarbazide (TSC, Sigma), was used in doses of 3 and 5 mg/kg. AOAA and TSC were dissolved in 0.1 ml of saline and injected intraperitoneally 1 hour before female presentation. The same doses of saline were administered to control animals under similar conditions. GABA receptors were blocked (9) by bicuculline (Serva) given in doses of 1 and 1.8 mg/kg. Prior to injection bicuculline was diluted in a drop of 0.1 N HCl and adjusted to the concentration required with distilled water, pH 3.5. Bicuculline (0.1 ml, SC) was administered at the moment of female presentation. Control animals received 0.1 ml of the solvent.

Statistical Analysis

Statistical analysis of the results was performed by two-way analysis of variance (ANOVA) and Student's *t*-test.

RESULTS

The levels of testosterone in peripheral blood plasma depended on the animal genotype, $F(2,37) = 49.04$, $p<0.001$. Initial testosterone levels were lowest in males of A/He strain and highest in CBA/Lac (Table 1). A 40-min presentation of a receptive female to a male induced appreciable activation of hypothalamic-pituitary-testicular complex, $F(1,37) = 6.11$, $p<0.001$. A 1.5–3.5-fold increase in testosterone levels in blood were observed within 40 min (Table 1) and the extent of the increase also depended on the animal's genotype, $F(2,37) = 3.40$, $p<0.04$.

GABA accumulation in peripheral tissues and the brain, induced by the intraperitoneal administration of AOAA, had no effect on resting testosterone levels in blood. However, it completely inhibited plasma testosterone levels of males in response to a female presentation. Furthermore, 40 min after female presentation, the mice of CBA/Lac strain which received the highest AOAA dose exhibited a decrease in the blood testosterone concentrations below initial control levels (Table 1). ANOVA showed that, under these conditions, plasma testosterone levels were influenced by both the mice genotype, $F(2,43) = 75.06$, $p<0.001$, and the elevation of GABA content, $F(1,43) = 10.17$, $p<0.002$; the effect of GABA depended on the animal hereditary peculiarities, $F(2,43) = 6.12$, $p<0.004$.

TABLE 1

EFFECT OF DRUGS INFLUENCING GABAERGIC MECHANISM ON PLASMA TESTOSTERONE LEVEL IN MALE MICE OF DIFFERENT GENOTYPES 40 MIN AFTER PRESENTATION OF A SEXUALLY RECEPTIVE FEMALE

	Mean Testosterone Level, nmol/l ± S.E.M.		
Treatment	A/He	CBA/Lac	BALB/c
Resting Level	5.3 ± 0.70(9)	10.2 ± 1.58(9)	9.8 ± 1.29(5)
Saline + SA	20.7 ± 5.50(6)	17.2 ± 2.87(7)	22.0 ± 5.29(5)
AOAA, 20 mg/kg	4.2 ± 0.32(8)	11.6 ± 2.24(8)	9.1 ± 1.09(8)
AOAA, 15 mg/kg + SA	6.7 ± 1.12(6)‡	4.9 ± 1.44(6)‡	10.2 ± 1.29(5)‡
AOAA, 20 mg/kg + SA	4.2 ± 0.70(10)†	2.5 ± 0.59(10)‡	10.9 ± 0.88(9)*
Resting level	5.3 ± 0.70(9)	10.2 ± 1.58(9)	9.8 ± 0.37(5)
Saline + SA	20.7 ± 5.50(6)	17.2 ± 2.87(7)	22.0 ± 5.29(5)
TSK, 5 mg/kg	4.9 ± 0.49(8)	9.1 ± 1.16(8)	10.2 ± 0.95(8)
TSK, 3 mg/kg + SA	10.5 ± 0.53(8)	15.0 ± 1.44(7)	17.5 ± 2.42(5)
TSK, 5 mg/kg + SA	13.7 ± 2.56(9)	14.0 ± 1.12(5)	15.4 ± 2.10(5)
Resting level	3.5 ± 0.67(5)	11.2 ± 3.05(8)	10.5 ± 1.47(5)
Vehicle + SA	22.1 ± 1.12(9)	18.5 ± 1.44(7)	23.1 ± 2.91(9)
Bicuculline, 1 mg/kg	4.2 ± 0.81(6)	11.2 ± 2.24(8)	11.2 ± 1.85(7)
Bicuculline, 1 mg/kg + SA	18.9 ± 1.86(8)	13.7 ± 2.17(8)	23.5 ± 4.76(8)
Bicuculline, 1.8 mg/kg + SA	22.8 ± 2.59(8)	17.5 ± 2.87(8)	24.9 ± 3.89((5)

SA: sexual arousal, AOAA: aminooxyacetic acid, TSK: thiosemicarbazide. The number of mice is in parentheses. *$p<0.05$, †$p<0.01$, ‡$p<0.001$ vs. saline + SA the same strain of mice.

It also should be noted that the decrease in GABA content which followed TSC administration did not affect either initial plasma testosterone levels, or this steroid's concentration during the course of a female presentation. The levels of plasma androgen following administration of a glutamate decarboxylase inhibitor was somehow lower in the presence of a female but did not differ significantly from those obtained in blood in the control group (Table 1).

The blockade of GABA receptors, induced by bicuculline administration, had no effect on the rise in plasma testosterone which occurred in the presence of a receptive female. Even the sublethal (1.8 mg/kg) dose of bicuculline (the doses of 2 mg/kg led to death in 60% of animals) did not affect the testosterone levels in peripheral blood (Table 1).

ANOVA showed that the lack of the effects of both a decrease in GABA content, $F(1,32) = 1.75$, $p>0.19$, or blockade of GABA-receptors, $F(1,35) = 0.43$, $p = 1.0$, on the blood testosterone levels in response to presentation of a female were independent of the animal's genotype, $F(2,32) = 0.15$, $p = 1.0$ and $F(2,35) = 0.48$, $p = 1.0$, respectively.

Emotional stress, induced by restriction of a male in the presence of a receptive female beyond a partition in the same cage suppressed plasma testosterone levels in a manner similar to that of AOAA (Fig. 1). It should be noted that in males with AOAA-induced GABA content elevation, the administration of bicuculline was accompanied by almost two-fold increase of testosterone levels in the presence of a receptive female. An even more prominent effect was revealed upon emotional stress. More

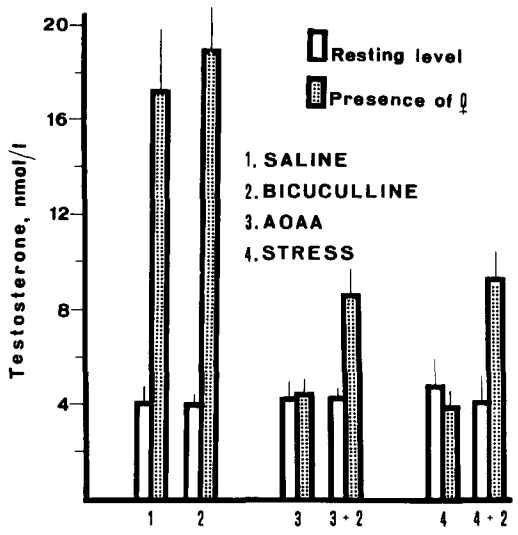

FIG. 1. Effect of bicuculline-sensitive GABA receptor blockade on plasma testosterone levels in male mice 40 min after presentation of a sexually receptive female under emotional stress or aminooxyacetic acid (AOAA) pretreatment. Each group consists of 6–9 mice.

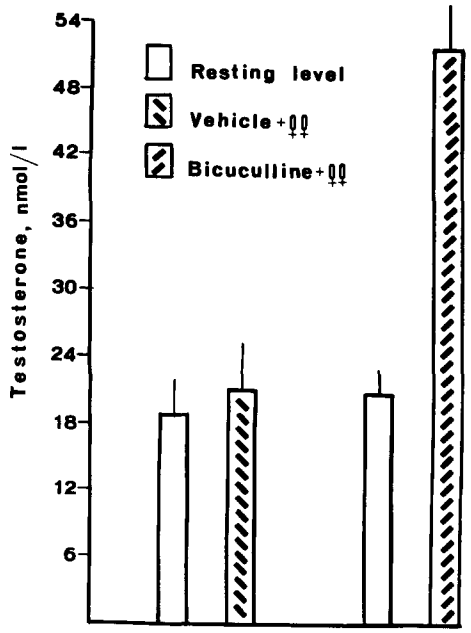

FIG. 2. Effect of bicuculline-sensitive GABA receptor blockade on plasma testosterone levels in a group of male mice 40 min after presentation of a group of sexually receptive females in the same cage. Each group consists of 6 animals. Each column represents mean ± S.E.M. of 4 groups of male mice.

than a two-fold increase of plasma testosterone levels were observed in mice following blockade of GABA-receptors by bicuculline (Fig. 1).

A 40-min presentation of six sexually receptive females to a group of males in the same cage resulted in development of emotional stress. The mean levels of corticosterone in blood of the males reliably and sharply increased from 152.9 nmole/l to 663.8 nmole/l. Under these conditions, the presence of receptive females provoked no elevation of testicular hormone. However, the administration of a GABA receptor antagonist to males at the moment of female presentation increased blood testosterone levels following emotional stress (Fig. 2).

DISCUSSION

The initial testosterone levels, as well as the extent of its elevation in blood induced by the presence of a sexually receptive female, depend on the male genotype. These results completely agree with our earlier observations (28,31).

The drug-induced accumulation of GABA in peripheral tissues and the brain inhibits the increase in plasma testosterone which occurs in response to presentation of a receptive female. However, neither a decrease in GABA concentration, nor the blockage of GABAergic receptors affected the endocrine function of testis. These results suggest that the GABAergic system participates in regulation of sexual activity only when activated by some processes which control the GABA system. Under normal resting conditions, this phenomenon does not seem to occur but it may be switched on in response to stress. An inhibitory effect of emotional social stress on the gonads and sexual behavior, induced by increasing density of populations, have been demonstrated earlier (7, 8, 26).

Hence, one may suppose that inhibition of sexual motivation following stress is determined by increasing the tone of the GABAergic system since plasma testosterone levels in response to female presentation has been shown to be blocked following GABA accumulation with a concomitant increase in GABAergic activity. Thus the inhibition of endocrine function of testis (revealed at increasing GABAergic tone under stress) can be considered as an adaptive response and as a mechanism which inhibits sexual behaviour following stress. To test this hypothesis, we studied some of the GABAergic processes which occur during stress.

If the elevation of GABA levels during stress inhibits sexual activity, administration of a GABA receptor antagonist must either facilitate or restore sexual activation under stressful conditions or when GABA concentrations are elevated. In fact, administration of bicuculline during emotional stress or when elevated GABA levels are induced by AOAA results in a two-fold increase of plasma testosterone levels after female presentation. Similar results also have been obtained for a group of males after presentation of a group of sexually receptive females in the same cage.

It should be noted that although emotional stress, in bicuculline-treated animals, is accompanied by an appreciable rise in sexual activity, the level of testosterone on blood increases to a lesser extent as compared to that obtained in the absence of stress. So, we cannot neglect participation of other GABAergic receptors which are insensitive to bicuculline (6,19) in the inhibitory effect of stress on plasma testosterone levels. Nevertheless, the antagonistic action of bicuculline on the effect of emotional stress and that of AOAA administration support the hypothesis that, following stress, GABAergic processes are involved in the inhibition of sexual arousal in males. It can be supposed that activation of GABAergic system is an adaptive response which, in natural conditions, controls sexual behaviour upon stress. GABAergic receptors sensitive to bicuculline seem to play a key role in this process.

There are evidences indicating that both acute (23,36) and prolonged (17) stresses result in an increase in activity of glutamic acid decarboxylase along with an elevation in GABA con-

centrations in some brain regions and nuclei. It was suggested that the effect of stress on GABAergic neurones was regulated primarily by alterations in the activity of the brain glutamic acid decarboxylase (23,36). Therefore, it can be assumed that an involvement of the brain GABAergic system in control of the hypothalamic-pituitary-testicular responses of male mice to females under stress conditions. On the other hand, GABA has been shown to play a role as a putative neurotransmitter in peripheral nervous system (20). Moreover, direct GABA effects on the gonads may occur since GABA has been identified in high concentration and a high density of GABA-receptors exist on membranes of gonadal cells (13,34). Moreover, recent in vitro experiments have shown that GABA may regulate testicular androgen production in rats (33). Since we administrated AOAA intraperitoneally, it is possible that GABA accumulates not only in brain but also in the testes where it can directly influence testicular function. This possibility requires further investigation.

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