EXPERIMENTAL BIOLOGY

Testosterone and Behavior: Involvement of the Hormone in Psychotropic Effects of Baclofen

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We studied the effect of baclofen ($GABA_B$ receptor agonist) on the behavior of male mice with different levels of anxiety in tests for social and sexual contact and on blood testosterone levels. The drug reduced testosterone level and behavioral reaction to an unknown male in intact animals and did not modulate the hormone level and social contacts in anxious mice. In the test with receptive female, baclofen reduced testosterone level and sexual motivation in intact males and did not modulate the hormone level and initial sexual interest in anxious mice. Parallelism in the development of behavioral and endocrine components of the reaction to social and sexual stimuli confirms possible involvement of testosterone in psychotropic effects of baclofen.

Key Words: *baclofen; $GABA_B$ receptors; testosterone; anxiety; sexual motivation*

Brain neurotransmitters play a key role in the regulation of animal behavior, according to modern views of neurophysiology. On the other hand, psychotropic effects are produced by many other bioactive substances, including classical hormones [10,14].

Androgens involved in the regulation of aggressive and reproductive behavior of males are best studied from the behavioral viewpoint [7,12]. Recent studies demonstrated anxiolytic effects of male sexual hormone testosterone (TS) [5,15]. Experiments on rats and mice showed that administration of TS facilitated neurotransmission through the $GABA_A$ receptor site and led to anxiolysis. Presumably, TS, like other neurosteroids with anxiolytic characteristics [8], possesses affinity to $GABA_A$ receptor site and this is the main or one of the mechanisms of its effect on anxiety level in animals.

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Another aspect of the hormone-neurotransmitter interactions is the involvement of GABAergic mechanisms in the regulation of blood TS content [9,11]. For example, baclofen ($GABA_B$ receptor agonist) modulates TS synthesis by the testicles [13] and activates the compensatory increment in TS level in case of its reduction [1]. It remains unclear whether the psychotropic effects of baclofen are TS-mediated or not.

We verified the hypothesis on possible involvement of TS in the behavioral effects of baclofen during social and sexual contacts of males with different levels of anxiety.

MATERIALS AND METHODS

Experiments were carried out on adult male C57Bl/6J mice aged 2.5-3.0 months (24-28 g). Behavioral model of chronic social stress was used: the males daily (for 20 days) experienced social defeats in intermale confrontations, which led to the development of an anxious status [3]. Intact males served as controls. Each group consisted of 10-12 animals.

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Behavioral activity of males in response to known and unknown partners (5+5 min) was studied in the wall test (quantitative evaluation of communicative activity of animals as the indicator of their anxiety [4]).

Behavioral reaction of males was studied in a 30-min test for sexual motivation (SM) (evaluation of the intensity and stability of behavioral reaction of a male to an estrus female of the same genotype placed behind a transparent wall with holes [3]) in a series of four 5-min sessions: 2 at the beginning and 2 at the end of the test period. At the end of the test, the males were decapitated and blood was

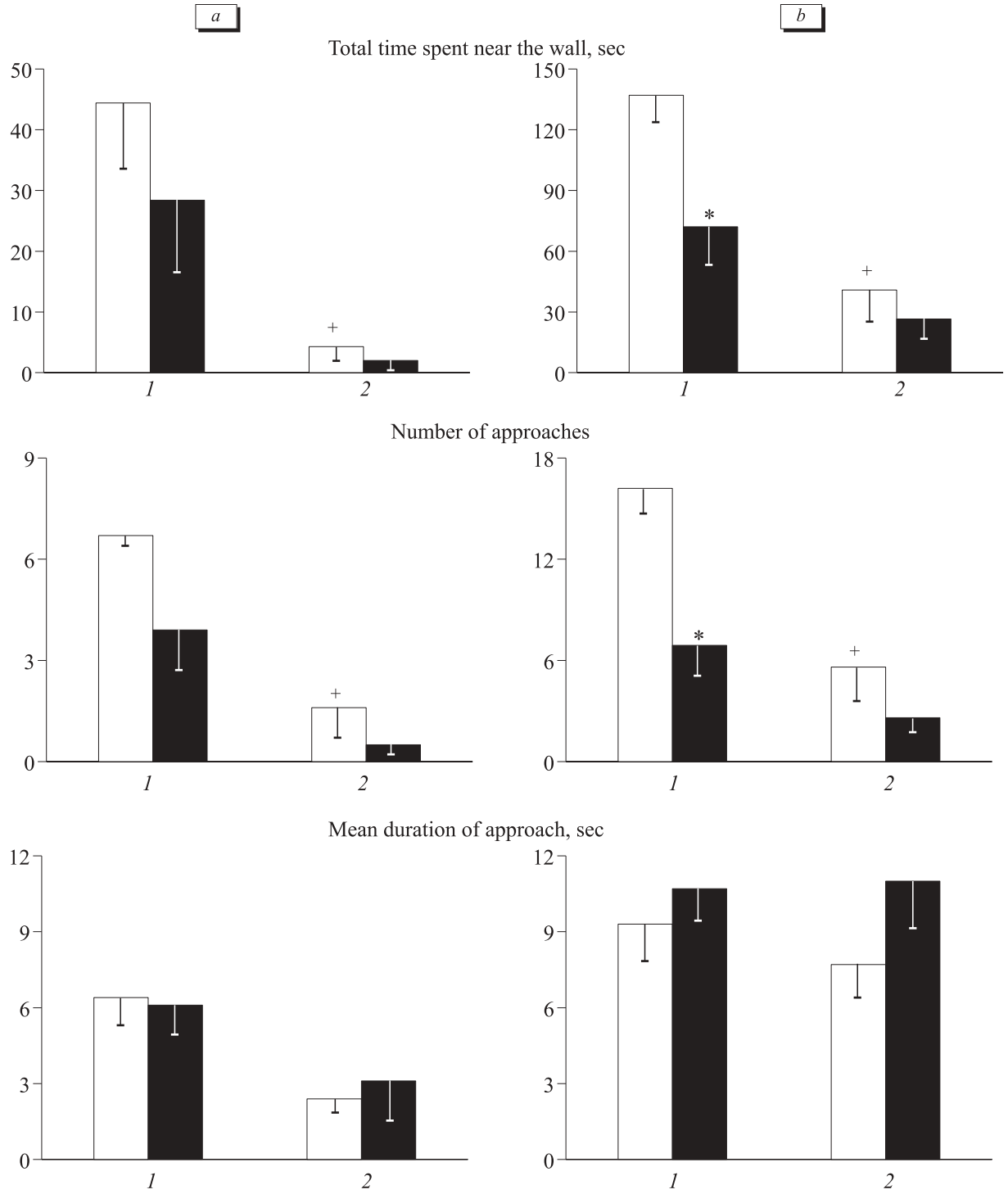

Fig. 1. Effect of baclofen on the behavior of males in the wall test. *a*) reaction to a known male; *b*) reaction to an unknown male. Here and in Figs. 2, 3: light bars: saline; dark bars: baclofen. *1*) intact; *2*) submissive animals. $p<0.05$ compared to: *injection of saline to intact animals; +intact animals, **test session 1.

collected for TS measurement. Behavioral parameters were recorded using Mouse software.

Baclofen (ICN) in a single dose of 5 mg/kg was injected intraperitoneally 40 min before behavioral testing.

Blood samples for the analysis of basal TS level were collected 40 min after injection of baclofen (or saline), samples for evaluating TS level stimulated by the female were collected after SM test. Plasma TS level was measured by enzyme-linked immunosorbent assay with standard reagents.

The data were statistically processed using nonparametric Mann—Whitney's *U* and Wilcoxon's *T* tests.

RESULTS

Chronically stressed males exhibited reduced behavioral reaction to known and unknown partners in the wall test (Fig. 1), which, together with other behavioral tests [4], indicates high anxiety. In parallel, submissive males had reduced basal blood TS levels in comparison with intact males (Fig. 2, *a*). These data were for a long time considered inessential for understanding of the anxiety phenomenon. However, after discovery of anxiolytic effects of TS [5,15] it appeared logic that reduced blood level of TS could serve as the factor triggering the development of anxious status in animals.

Injection of baclofen to intact mice reduced basal TS level and in parallel, inhibited behavioral reaction in the wall test only in an anxiogenic situation (contact with an unknown partner). Reaction to a known male, not associated with threat, did not change. Injection of $GABA_B$ agonist to anxious males did not modify low content of TS and social reaction to the known and unknown test males behind the wall. It seems that chronic stress situation forming the anxious status of animals promoted total desensitization of the GABAergic mechanisms, which explains the absence of apparent effects of this drug dose.

On the whole, the results indicate parallel regulation of the anxious status and hormone concentration through $GABA_B$ receptors or, which seems more plausible, indirect effect of baclofen on mouse anxiety mediated through modulation of the blood TS content.

In the SM test (sensitive to drug treatment) anxious males exhibited decreased primary reaction to a receptive female (decreased number and duration of approaches to the wall) and its exhaustion with time (reduction of total duration of contacts with the female from session to session). Sexual motivation of intact males was stable [2] (Fig. 3).

Blood level of TS stimulated by the presence of the female was higher in intact males than in anxious ones (Fig. 2, *b*). It seems that initially higher TS content in intact mice corresponded to more active behavioral reaction to a female. Reduced TS level in submissive animals correlated with less intense SM. Presumably, a neuropsychoendocrine mechanism with positive feedback is functioning in a situation of contact with the female, when the intensity of initial behavioral reaction is proportional to the content of TS. This behavioral reaction, in turn, stimulates the production of an additional amount of TS, which supports the developing behavior. The data on positive correlation between the parameters of contact with the female during the first 10 min of the test and subsequent level of TS in the blood confirm this hypothesis [6].

Injection of baclofen to intact males before presentation of a receptive female provoked a re-

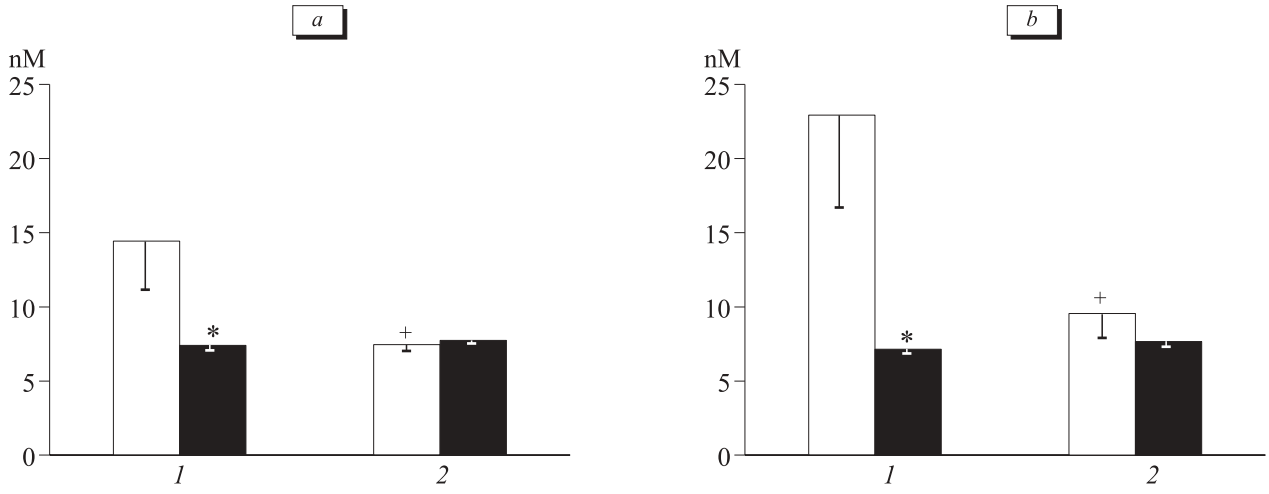

Fig. 2. Effect of baclofen on plasma TS level. *a*) basal level; *b*) in the presence of a female.

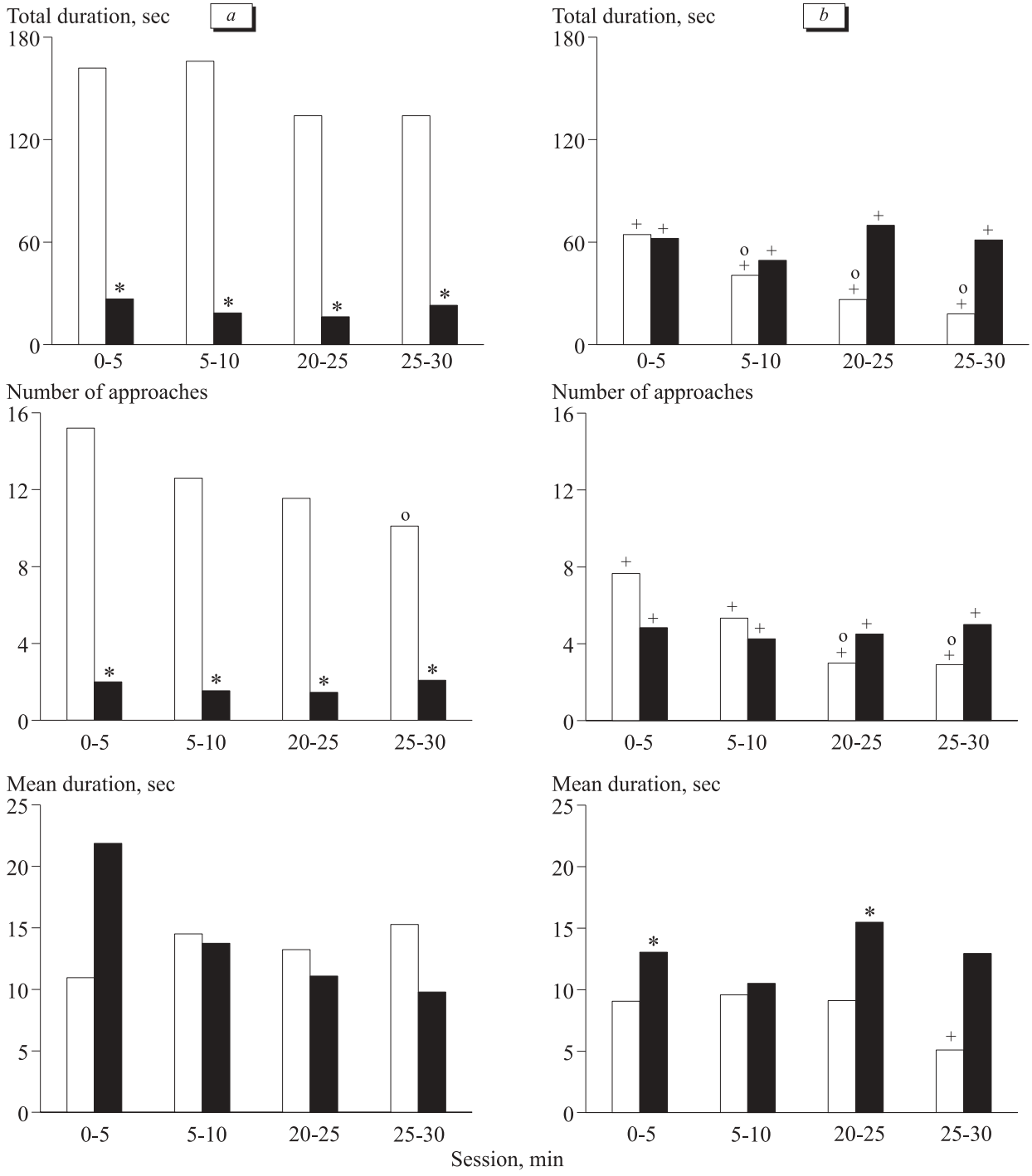

Fig. 3. Baclofen effect on SM of intact (*a*) and submissive (*b*) males. °$p<0.05$ compared to 0-5 min session in the same animals.

duction of sexual interest. The latent period of the first approach to the wall increased (saline: 14.5± 5.2 sec; baclofen: 146.0±44.5 sec; $p=0.03$), number of approaches and duration of contact with the female decreased. Blood TS level also reduced in intact males after baclofen treatment. This clear-cut parallelism of baclofen effects on the behavioral and endocrine components of the response to a sexual stimulus, similarly as in the situation with social contact with an unknown male, indicates that its effect on behavior is mediated through TS.

In submissive males baclofen injection did not change the initial reaction to the female and did not modulate TS level. On the other hand, SM of anxious mice injected with baclofen was not exhausted during the second half of the test, though by

the absolute values these parameters remained lower than in intact animals. The absence of SM exhaustion without appreciable effect of the drug on TS level once more confirms the intricate structure of behavioral reactions, which are modulated by baclofen by other mechanisms, in addition to the hormonal one.

These results give us more profound understanding of the possible mechanisms of behavioral regulation. Drug effects on social and sexual behavioral reactions, mediated through TS, are shown as exemplified by baclofen ($GABA_B$ receptor agonist). Experimental data are in line with modern concepts of TS as an active psychotropic substance with obvious anxiolytic effects.

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