GENETICS

Effect of Acute and Chronic Buspirone Administration on Communicativeness of Mice with Experience of Defeats in Social Conflicts

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We studied the effect of 5-HT_{1A} receptor agonist buspirone on behavior of male C57Bl/6J mice in the "partition" test, which reflects communicativeness of animals. Single administration of buspirone (1 mg/kg) to intact mice and animals experienced defeats in 20 intermale confrontations impaired their communicativeness, especially in intact animals. On the contrary, administration of buspirone (1 mg/kg) to losers starting from day 5 of intermale confrontations for 2 weeks produced a positive effect and prevented impairment of communicativeness by day 20 of confrontations. The role of brain 5-HT_{1A} receptors in these processes is discussed.

Key Words: *mice; intermale confrontations; buspirone; communicative behavior*

Sociability, including social interactions and communicative behavior of animals, is considered to be an essential form of contacts in mice and rats [6]. In fact, demonstration of any type of behavior in animals is not only genetically determined, but also is modulated by environmental conditions. The pathogenic influence of long-term intermale confrontations on behavior of C57Bl/6J mice experiencing everyday defeats in these confrontations was many times demonstrated in our experiments [1,10]. These mice develop generalized anxiety manifesting in numerous tests. Communicativeness estimated in the "partition" test decreases in parallel with increase in anxiety [11]. The inhibitory action of enhanced anxiety on manifestation of normal sociability in animals was also demonstrated by other authors [5,8].

Since behavioral disturbances developed in male mice as soon as after 3 days of intermale confrontations and persisted for a long time, while after 20 days the mice developed depression-like state in addition to increased anxiety and decreased communicativeness, we can speak about the development of anxious depression in these animals [1], which is a model of such pathology caused by social stress in humans. Our numerous studies of behavior, neurochemical changes in the brain of loser mice, and pharmacological data confirm adequacy of this model. It is obvious that the search for possible pharmacological correction of changes in behavior developing at the early stages of social stress aimed at prevention of further severe pathology, is an important problem. Here we attempted to prevent deterioration of communicativeness in C57Bl/6J mice developing as a result of everyday defeats in intermale confrontations [1], by administration of anxiolytic buspirone widely used in clinical practice, particularly for the treatment of people with anxiety-depressive disorders [2]. Taking into account the fact that single and chronic buspirone administration [9,12,14] showed different and sometimes oppo-

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site effects on animal models of anxiety, we compared the effects of single administration of buspirone to animals after 20 social defeats and chronic administration starting from day 5 of intermale confrontations.

MATERIALS AND METHODS

C57Bl/6J mice were kept and bred in standard vivarium conditions of Institute of Cytology and Genetics of Siberian Branch of the Russian Academy of Sciences at 12:12 h (light:darkness) light schedule with free access to food (pelleted forage) and water. Mature male mice weighing 26±1 g and aging 2.5-3 months were used in the experiments. The animals were placed into experimental chambers 28×14×10 cm divided into two equal compartments with a perforated partition, one mouse per compartment. Under these conditions, the mice could see, hear, and feel the smell of each other (sensory contacts), but no physical interaction was possible. After 2 days of habituation to the new environment, the animals were subjected to intermale confrontations in the afternoon (15.00-17.00 h) according to the sensory contact model [10]. The lid of the cage was replaced with transparent plexiglas cover and 5 min later (time needed for activation of mice and habituation to new light conditions) the partition was removed for 10 min, which led to agonistic interaction (intermale confrontation). The experience of defeats exhibited by one mouse from each pair during the first 3 days was consolidated thereafter upon repetitive conflicts with partners who demonstrated aggressive type of behavior. To this end, the loser after the confrontation was placed into unfamiliar cage containing another aggressive male behind the partition. During the entire experiment, aggressive males were kept in their cages. If intense attacks of aggressive partner in the course of agonistic conflicts continued for more than 3 min, the confrontation was stopped and the partition separating the mice was returned back on its place. As a result, we obtained a group of animals with everyday experience of defeats in confrontations over 20 days (losers T20).

The effect of partial 5-HT_{1A} receptor agonist buspirone on behavior of losers was determined for two modes of administration, single and chronic.

The effect of single administration of two buspirone doses (1 and 10 mg/kg; Sigma Chemical Co.) on behavior of intact males was preliminary studied, because buspirone in a dose of 3 mg/kg or higher can significantly affect locomotor activity of experimental animals [9,12]. Male mice were placed into individual cages 28×14×10 cm for 3 days; this procedure eliminates the experience of group interactions and the effect of social isolation does not develop. Losers T20 received single dose of buspirone selected on the basis of preliminary data on intact individuals. In both cases, the animals were randomly divided into two groups intraperitoneally receiving buspirone or vehicle (physiological saline). The drugs were injected 30 min before behavioral test.

The effect of chronic buspirone administration on behavior was investigated in a special group of losers obtained by the method of sensory contact [10]. To this end, all losers were divided into two groups: group 1 received buspirone every morning starting from day 5 of intermale confrontations for 14 days, group 2 mice received vehicle; intermale confrontations were carried our in the afternoon. Buspirone was administered in the same dose as for single administration. Each group consisted of 11-14 mice. Behavior of losers was estimated after 10 (losers T10) and 20 (losers T20) confrontations and 5-and 14-fold buspirone administration, respectively. Behavior of losers after buspirone administration was compared with behavior of losers receiving vehicle. In addition, behavior of T10 and T20 losers was compared with behavior of males after 4 confrontations and without drug/vehicle administration (losers T4).

The mice were examined in the "partition" test, which allows estimating animal communicativeness by behavioral activity near the partition in response to demonstration of partner placed in the other compartment of the cage. The number of approaches to the partition (and/or behavior towards it), total time spent near the partition, and a derived index, mean time of one approach, were determined. Parameters were recorded over 10 min: the first 5 min in response to a familiar partner (aggressor) behind the partition, the next 5 min in response to unfamiliar partner (group male). Quantitative analysis of frequency and duration of behavioral acts was performed using Etograph device.

The data were compared by nonparametric Mann–Whitney *U* test and Wilcoxon T test using standard Statistica 6.0 software. The results are presented as *M*±*SEM*.

RESULTS

The effect of buspirone on communicative behavior of intact animals in the "partition" test depended on its dose (Fig. 1). Buspirone in a dose of 1 mg/kg produced significant reduction of time spent near the partition in response to familiar partner and mean time of one approach in response to familiar and unfamiliar partner. The number of approaches and behavioral acts towards partition remained unchanged. These shifts attest to a decrease in animal communicativeness. Buspirone in a dose of 10 mg/kg change all analyzed parameters, which attested to a decrease in not only communi-

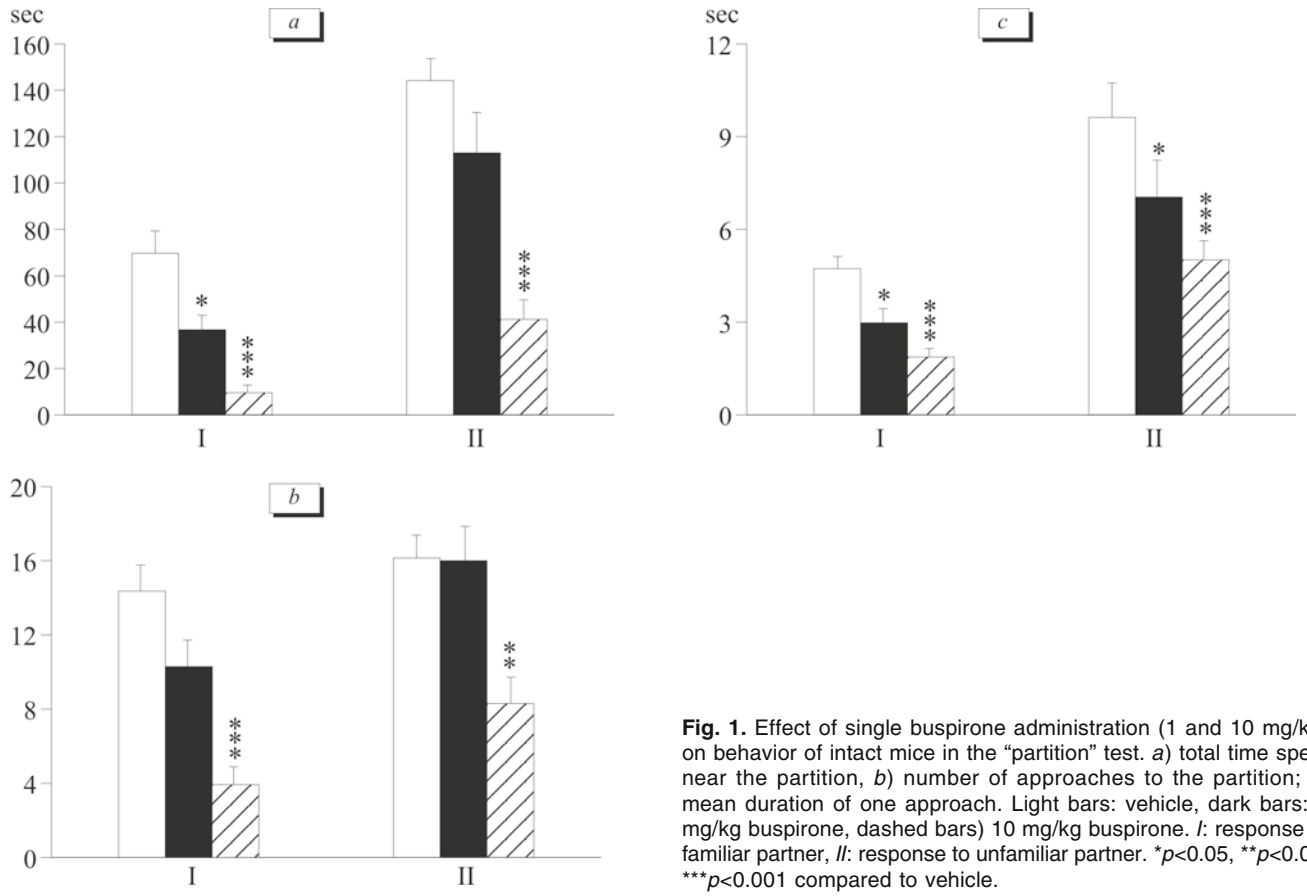

Fig. 1. Effect of single buspirone administration (1 and 10 mg/kg) on behavior of intact mice in the "partition" test. *a*) total time spent near the partition, *b*) number of approaches to the partition; *c*) mean duration of one approach. Light bars: vehicle, dark bars: 1 mg/kg buspirone, dashed bars) 10 mg/kg buspirone. *I*: response to familiar partner, *II*: response to unfamiliar partner. *p<0.05, **p<0.01, ***p<0.001 compared to vehicle.

cativeness, but also locomotor activity. Keeping in mind this fact and known data on negative effect of 3 mg/kg buspirone on motor activity [9,12], the dose 1 mg/kg was chosen for further experiments. In contrast to intact animals, effect of buspirone (1 mg/kg) on losers after 20 days of defeats was less pronounced (Table 1). The drug significantly decreased the derived index, mean time of one approach, in the response to unfamiliar partner.

Thus, the effect of the same dose of drug (1 mg/kg) proved to be different in intact mice and animals in a pathological condition, more pronounced in the former and weaker in the latter. According to our previous data, mice experiencing defeats in intermale confrontations for 20 days demonstrate dynamic changes in activity of the brain serotoninergic system [1]. At the initial stages, after 3-10 intermale confrontations, changes are predominantly observed in meta-

TABLE 1. Effect of Single Administration of Buspirone (1 mg/kg) on Behavior of Losers T20 in "Partition" Test

Parameters of behavior		Losers T20	
		vehicle	buspirone
Number of approaches to partition	familiar partner	4.83±1.30	2.42±1.08
	unfamiliar partner	5.33±1.44	6.92±2.25
Duration, sec	familiar partner	16.67±4.12	6.50±2.83
	unfamiliar partner	37.25±8.67	30.08±11.18
Mean time, sec	familiar partner	2.47±0.57	1.86±0.60
	unfamiliar partner	5.83±1.10	2.64±0.64*

Note. *p<0.05 compared to vehicle.

bolic indices of brain serotoninergic system: content of serotonin and 5-hydroxyindolylacetic acid and activity of enzymes of the transmitter synthesis and degradation. More prolonged experience of defeats in losers in intermale confrontations (20-30 days) decreased the sensitivity of postsynaptic 5-HT_{1A} receptors, which was demonstrated by pharmacological methods and radioligand binding assay [1]. The levels of serotonin and its metabolite in many brain structures were comparable to control levels in unstressed animals. Reduced sensitivity of 5-HT_{1A}-receptors in losers T20 is probably responsible for less pronounced effect of single buspirone administration on behavior.

Chronic treatment with buspirone produced a positive effect on communicative behavior of losers T20 in the "partition" test by the end of the treatment period (after 14 days; Table. 2). The number and time of partition approaches in response to unfamiliar partner significantly increased in losers T20 compared to losers receiving vehicle (horizontal comparison). Moreover, activity near the partition in losers T20 by the end of the second week was equal to that in losers T4 (vertical comparison), which also attested to

TABLE 2. Effect of Chronic Preventive Buspirone Administration (1 mg/kg) on Behavior of Losers T20 in "Partition" Test

Parameters of behavior		Losers T4	
		without administration	
Number of approaches to partition	familiar partner	7.36±1.06	8.00±0.83
	unfamiliar partner	7.82±1.43	9.92±1.10
Duration, sec	familiar partner	59.64±11.02	56.42±11.16
	unfamiliar partner	103.18±20.99	110.75±14.27
Mean time, sec	familiar partner	8.08±0.62	6.83±1.15
	unfamiliar partner	10.92±1.98	12.61±2.02
		Losers T10	
		vehicle	buspirone
Number of approaches to partition	familiar partner	6.45±1.22	6.08±1.03
	unfamiliar partner	9.27±1.40	9.00±1.34
Duration, sec	familiar partner	24.18±5.10^{+}	30.50±14.35
	unfamiliar partner	75.82±12.16	85.25±16.10
Mean time, sec	familiar partner	3.88±0.71^{++}	4.29±1.08
	unfamiliar partner	9.13±1.45	10.09±2.73
		Losers T20	
		vehicle	buspirone
Number of approaches to partition	familiar partner	6.18±1.03	9.83±1.64
	unfamiliar partner	6.55±1.31	11.17±1.39*
Duration, sec	familiar partner	25.36±5.72^{++}	49.08±9.30
	unfamiliar partner	60.55±10.34^{+}	123.50±16.39**
Mean time, sec	familiar partner	3.95±0.43^{++}	4.91±0.50
	unfamiliar partner	10.40±1.12	10.37±1.25

Note. *p<0.05, **p<0.01 compared to vehicle (horizontal comparison); ^{+}p<0.05, ^{++}p<0.01 compared to the corresponding parameter in losers T4 (vertical comparison).

the positive effect of buspirone. At the same time, the time spent near the partition by losers receiving saline gradually decreased with increasing the number of intermale confrontations and in response to unfamiliar partner at T20 stage. The mean time of one approach towards the partition in response to familiar partner, decreased. Thus, buspirone attenuated the negative influence of intermale confrontations and maintained communicativeness in losers at the level observed at early stages of animal interaction. Since mouse behavior in the "partition" test to a large extend depends on their anxiety [1], not only the procommunicative effect of buspirone, but also its anxiolytic action can be suggested. This is also supported by the data on positive effect of buspirone on behavior of losers in elevated plus maze test [1], which is considered to be a classical test for anxiety estimation.

Thus, analysis of our findings revealed interesting facts: single administration of buspirone had a negative effect and chronic administration produces a positive effect on animal communicativeness. It should be noted that chronic buspirone administration was started from day 5 of intermale confrontations, *i.e.* against the background of enhanced activity of the brain serotoninergic system [1]. Under these conditions, high concentration of both serotonin and 5-HT_{1A} receptor antagonist buspirone in the synaptic cleft can accelerate desensitization of somatodendritic 5-HT_{1A}-autoreceptors, as it was shown by other authors [3,4]. This is desensitization of 5-HT_{1A}-autotoreceptors, which other authors associate with anxiolytic effect of chronic azapirone administration [3,13].

However, it cannot be excluded that the effect of buspirone is caused by contribution of other receptors, since it is known that buspirone is not only a partial agonist of 5-HT_{1A}-receptors, but also possesses antagonistic activity towards brain dopamine D_2 receptors [7]. In addition, the main buspirone metabolite l-(2-pirimidinyl)-piperazine, which is rapidly formed and accumulated in the brain during chronic buspirone administration, possesses properties of α_2-receptor antagonist [7]. It is known that pharmacological blockade of D_2 receptors reduces, while α_2-receptor blockade increases the extracellular content of serotonin [15]. Therefore, the effects of buspirone can be modulated via these receptors. This leads to normalization of the level of functional serotonin in the synaptic cleft, which probably determines the anxiolytic effect of buspirone [1] and, as it as shown in these experiments, prevents reduction of communicativeness in mice even against the background of continuing intermale confrontations.

It should be noted that the positive effect of buspirone was achieved by prolonged administration to mice under conditions of continuing social defeat stress. Thus, we demonstrate the protective effect of buspirone, which prevents deterioration of communicativeness in animals. This is of particular importance in view of the fact that efficacy of buspirone was demonstrated in patients with severe pathology [2] in the absence, to the extent possible, of stressing factors, which provoked the pathological condition.

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