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EFFECT OF VASOPRESSIN V1B RECEPTOR ANTAGONIST, SSR149415, ON ANXIETY-LIKE BEHAVIOR AND LEWIS LUNG CARCINOMA METASTASIS IN MICE

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***Aim*: To study the effect of new vasopressin V1b receptor antagonist, SSR149415, on anxiety-like behavior and Lewis lung carcinoma metastasis in the anxious adult male mice of C57Bl/6J strain. This type of receptors was thought to act as potential targets mediating the effect of negative psychoemotional state on tumor progression. *Methods*: Anxiety-like psychoemotional state of the animals was produced using chronic social conflict model. Used behavioral tests were elevated plus-maze, social interaction test and open field test. Tumor cells were administrated on background of double or sixfold SSR149415 injections and the number of metastases in the lung were calculated 17 days later. *Results*: SSR149415 reduced the anxiety-like behavior measured in the elevated plus-maze and social interaction tests and did not affect locomotor activity in the open field test. Double and sixfold administration of the compound to such mice before and after inoculation of the tumor cells produced no effect on the metastasis rate. *Conclusion*: vasopressin V1b receptor is involved in the mediation of anxious behavior of animals but is not involved in the mechanism underlying the influence of negative psychoemotional state on Lewis lung carcinoma metastasis.**

Key Words: anxiety, Lewis lung carcinoma, metastasis, vasopressin receptor, SSR149415

The effect of psychoemotional state on tumor progression is an established phenomenon although its underlying mechanisms remain elusive. Three decades ago it was demonstrated that rearing of animals in overcrowded conditions and subsequent activation of the stress system of organism can significantly change the incidence of tumors and the number of tumor metastatic nodules [22]. The study of two types of transplantable tumors carried out in mice showed that animals with anxiety-like behavior induced by rearing in stressful conditions have twice as much of experimental metastases as intact mice [10, 16], while anxiolytic drug diazepam reduces the number of metastases [9].

Hypothalamic hormone vasopressin potentiating the action of corticoliberin can increase the secretion of the adrenocorticotropic hormone and subserves significantly to the formation of negative stress-induced psychoemotional states [13, 23]. Distribution of vasopressinergic fibers and receptors in the limbic structures of the brain suggests the involvement of this neuropeptide in emotional responses to stress [11]. The animals with depression semiotics and patients with major depression were shown to have an increased plasmatic level of vasopressin. In both cases administration of antidepressants normalizes the hormone level [4, 12]. Rats selected for high anxiety behavior exhibit innate elevated expression of vasopressin mRNA and hypothalamic receptor binding [18]. Exogenous intraventricularly administered vasopressin produces anxiogenic effect while its antagonists alleviate the symptoms of anxiety [2, 7]. Clinically effective anxiolytic drugs reduce the expression of the vasopressin precursor gene [23].

In the experiments with Lewis lung carcinoma (LLC) metastasis, administration of vasopressin was shown to increase the number of metastatic nodules in the lungs of mice [1], similarly as it occurs in animals with an impaired psychoemotional state. Still it is so far not clearly how the mechanisms of vasopressin action on metastasis and psychoemotional state interrelate.

Physiological action of vasopressin is known to be mediated by receptors of three types: V1a, V1b, and V2. The receptors of the former two types are widespread in the CNS structures [8, 20] and are involved in anxious behavior [5]. Receptors of V1a type are also present on unstriped muscles of the brain vessels and V1b receptors — on corticotroph pituitary cells to implement the effect of vasopressin on the stress axis of organism [21]. Receptors of both types are sensitive to emotional stress. Anxiety-like behavior in animals correlates with enhanced V1-receptor binding in the CNS [12]. Addition of antisense mRNA of V1a receptor to mice results in a pronounced alleviation of anxiety-like behavior and impairment of social recognition. Chronic immobilization stress increases V1a mRNA level in rat brain [17].

Recently synthesized specific vasopressin V1b-receptor antagonist, SSR 149415 [7] provides the opportunity for distinguishing the contribution of receptors of this type to increasing of tumor metastasis in animals with pronounced anxiety-like behavior, which has become the purpose of this study.

MATERIALS AND METHODS

Animals. Male C56B1/6J strain mice, weighing 25–28 g, housed in standard conditions of the vivarium of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences

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Abbreviations used: CNS – central nervous system; EPM - elevated plus-maze; LLC – Lewis lung carcinoma.

were used. All experimental procedures with animals were carried out in accordance with the international rules established by the European Community Council Directive (86/609/EEC).

Anxious pattern of mice behavior was produced using a 10-day chronic social conflict model [15].

Behavior Evaluation Methods

The elevated plus-maze (EPM). This method is used to measure anxiety-like behavior of the mice placed in unfamiliar environment [24]. The four-minute experimental sessions were recorded with video camera. The conventional behavioral measures were recorded with the use of the "Mouse" software package (Institute of Cytology and Genetics, SB RAS). The most significant anxiety-related measures included time spent in the central area of the maze, time in the closed arms and time in the open arms and area (sum of central area and open arms times), and latent period of closed-arm entries. Exploratory behavior measures were the number of dipping postures with head below the level of the maze and stretching postures.

The "Partition" social interaction test. This test is used to obtain quantitative measures of sociability (communicativeness) of the animals relative to the level of anxiety [6]. The behavior of the mice in anxiety-like state was assessed in response to a known aggressive male and a strange male placed by turns in the neighboring compartment of the home cage across a perforated transparent partition. During the 6-minute test periods (3+3), the number of approaches and time at the partition were recorded for the test male and the derived measure — average time at the partition per approach — was calculated as an indirect measure of communicative motivation of the mice.

Open field test. This test is used to evaluate locomotor and exploratory activities. Three-minute test sessions were recorded with video camera. The behavior of the mice was recorded and calculations were made to find the distance, average speed, immobility time, number of upright postures and explorations of the setting holes as well as the time in the central and peripheral zones of the setting.

Oncological Experiments. Lewis lung carcinoma cells were inoculated into the tail vein (0.5 ml of suspension, 170 thousands cells/mouse). Seventeen days later the mice were decapitated; the lungs were fixed in 10% formalin solution. The number of metastatic nodules was calculated under a microscope with 8-fold magnification. Two experiments were performed:

double administration of SSR149415 — a day before and on the day of the tumor cell inoculation;

sixfold administration of SSR149415 — once a day during 6 days. Tumor cells were inoculated on the fourth day of SSR149415 injections.

Pharmacology. Vasopressin V1b receptor antagonist, SSR149415, (10 mg/kg, suspended in 0.6% methylcellulose and 0.5% Tween solution) was administrated once 1 hour before the behavioral tests. Also, in the day of tumor cell inoculation, the compound was administrated 1 hour before LLC cell injection too. Since the drug is known to be equally active at intraperitoneal and oral administration [7], both methods were used in our experiments. The vehicle in equal volumes was administered by the same methods to control mice.

Statistical Processing. The results were analyzed with the use of Student's t-criterion using STATISTIKA 6.0 software package. Each experimental group consisted of 11–15 animals.

RESULTS

Behavioral tests

Elevated plus-maze. Intraperitoneal administration of SSR149415 significantly reduced the level of anxiety-like behavior in animals (Table 1). The mice spent almost twice as less time in the EPM closed arms and thrice as much time in the open areas as compared to control. The latent period for entries into the closed arms also grew significantly. The time of staying in the center of the maze increased 2.5 times versus the control, which could be interpreted as a reduced speed of "decision making" on the backdrop of the drug action. Exploratory activity measures — dipping postures with heads below the maze and stretching postures showed no reliable difference from the control values mainly due to a considerable scattering of data.

Table 1. Effect of vasopressin receptor antagonist, SSR149415, on the behavior of male mice with anxiety-like behavior in elevated plus-maze test

	Anxious animals + vehicle (15)	Anxious animals + SSR149415 (13)
Time in closed arms, sec	195.53 ± 13.96	109.92 ± 25.11 **
Latent period of closed arm entry, sec	19.20 ± 7.31	78.46 ± 25.50 *
Time in open arms, sec	5.60 ± 2.73	29.69 ± 18.06
Time in the center of the maze, sec	38.00 ± 13.34	97.77 ± 22.90 *
Time in open areas, sec	43.60 ± 13.76	127.46 ± 25.85 **
Number of dipping postures	2.87 ± 0.82	5.46 ±1.14
Number of stretching postures	0.73 ± 0.41	2.69 ± 1.00

Note. *$p<0.05$; **$p<0.01$ compared to animals with anxiety, administration of the vehicle.

"Partition" social interaction test. Oral administration of SSR149415 produced no effect on the main measure of communicativeness — time spent by the mice at the partition exploring the smell of a male placed in the neighboring compartment (Table 2). Nonetheless, the drug administration to male mice with anxiety-like symptoms in the situation with unfamiliar male behind the partition doubled the derived measure — the time at the partition per approach — as compared to control mice suggesting an enhancement in mice motivation for communication with a conspecific and reduction of anxiety-like behavior following the administration of SSR149415.

Table 2. Effect of vasopressin receptor antagonist, SSR149415, on the behavior of male mice with anxiety-like behavior in the social interaction test

	Anxious animals + vehicle (15)	Anxious animals + SSR149415 (11)
Familiar male:		
Number of interactions (N)	4.23 ± 0.43	3.90 ± 0.85
Time of interactions (T), sec	41.31 ± 7.73	38.70 ± 6.12
Average interaction time (T/N), sec	9.69 ± 1.59	12.44 ±2.30
Unfamiliar male:		
Number of interactions	4.13 ± 0.75	3.09 ± 0.77
Time of interactions, sec	67.47 ± 12.17	69.73 ± 10.79
Average interaction time, sec	17.37 ± 1.72	34.76 ± 8.15 *

Note. *$p<0.05$; **$p<0.01$ compared to animals with anxiety-like behavior receiving the vehicle.

Open field test. Oral administration of SSR149415 did not affect locomotor or exploratory activity of animals with anxiety-like behavior in the test (Table 3).

Table 3. Effect of vasopressin receptor antagonist, SSR149415, on the behavior of male mice with anxiety-like behavior in open field test

	Anxious animals + vehicle (15)	Anxious animals + SSR149415 (11)
Passage distance, cm	290 ± 37	308 ± 42
Immobility time, sec	49.3 ±10.4	62.4 ± 9.6
Speed (distance/time in motion, cm/sec)	2.2 ± 0.2	2.5 ±0.3
Time in the central area, sec	50.2 ± 10.2	36.0 ± 7.4
Time in peripheral area, sec	130.8 ± 10.1	142.2 ±7.6
Number of holes explored	6.4 ± 1.1	5.3 ± 1.1
Number of vertical postures	3.1 ± 0.7	2.7 ± 0.7

Metastasis

As a rule, the number of LLC metastatic nodules in the lungs positively correlates with the level of anxiety in animals. In our experiments this correlation was reliably reproduced in the second experiment (Table 4).

Table 4. Effect of vasopressin receptor antagonist, SSR149415, on metastasis of Lewis lung carcinoma in the lungs of male mice with anxiety-like behavior

	Number of metastatic nodules
Experiment 1: Intact animals + vehicle	9.9 ± 1.7 (14)
Animals with anxiety-like behavior + vehicle	12.8 ± 1.34 (15)
Animals with anxiety-like behavior + SSR149415	14.54 ± 1.93 (13)
Experiment 2: Intact animals + vehicle	8.2 ± 1.3 (11)
Animals with anxiety-like behavior + vehicle	14.7 ± 2.2 (15) *
Animals with anxiety-like behavior + SSR149415	15.0 ± 2.2 (14)

Note *$p<0.05$ as compared with intact animals receiving the vehicle.

In the first experiment, after double intraperitoneal administration of SSR149415 to mice with anxiety-like behavior the number of metastatic nodules did not change as compared with administration of vehicle to control group.

In the second experiment, after six oral administrations of SSR149415 the number of metastatic nodules did not differ from control values.

DISCUSSION

Possible influence of emotional state of an individual on the course of tumor progression has been of interest exclusively for practicing psychologists [3, 25]. In the literature under discussion is whether and to what degree psychotherapy can influence the treatment of patients with tumors. The opinions on this topic seem to be equally divided. We failed to find any biochemical studies aiming to elicit the correlation between psychoemotional state of patients (including the activity of hypothalamus-pituitary-andrenal axis and vasopressinergic systems) and the efficacy of anticancer therapy.

The studies of the last decade demonstrated that vasopressin is a principal regulator of emotional behavior. Studies in humans support the co-activation of the central vasopressinergic system at anxiety and depression disorders [23] while clinically effective anxiolytic drugs and antidepressants reduce the expression of the vasopressin gene in the CNS [4]. It is vasopressin that provokes hyperactivation of the hypothalamus-pituitary-adrenal axis under chronic emotional stress by acting on pituitary corticotrophs through V1b receptors [14].

The leading role of vasopressin in behavioral phenomena of anxiety and depression and in stress-induced diseases was also demonstrated on animal models [18]. The Brattleboro rates deficient for vasopressin gene demonstrate elements of depression-like behavior [19]. In our experiment with mice a single subcutaneous administration of vasopressin caused behavioral reactivity of animals in the open field test [1].

In our experiments anxiety-like behavior was formed during 10 days by means of daily 10-minute agonistic interactions with a stronger male who was kept during the rest of the day in the neighboring compartment of the cage behind a perforated transparent partition. This procedure is sufficient to form a stable anxiety-like state in experimental animals [15]. Administration of V1b receptor antagonist, SSR149415, resulted in a visible reduction of anxiety level in the elevated cross-maze test and an increase of motivation in animals with anxiety-like behavior for social interaction with a strange male in the partition test. Therefore, in our experiments we reproduced the results confirming the anxiolytic effect of the drug [7]. After injection of vasopressin antagonist no changes in locomotor activity were recorded.

Tumor cells were inoculated 1–2 days after the last fighting. Administration of SSR149415 was made in the 1st experiment a day before and tumor cell administration day; in the 2nd experiment — 3 days before, during carcinoma cells inoculation day and two next days. The hours and days immediately after inoculation are thought to be a sensitive period for tumor cells dissemination and future growth of metastatic nodules. During this period, double and sixfold administration of SSR149415 did not change the increased intensity of Lewis lung carcinoma cells metastasis in animals with anxiety-like behavior irrespective of the route of drug administration. These results provide more assurance to claim that the previously found enhancing effect of a single vasopressin administration on the process of malignant metastasis is mediated not by V1b receptors. In other words, the V1b receptors are not involved in tumor cell dissemination and growth and the hormone action is not due to its psychotropic behaviorally inhibiting effect.

In this situation V1a receptors preserve their appeal to researchers as possible mediators of vasopressin effect on metastasis. Receptors of this type are located in different regions of the brain and blood vessels and together with V1b receptors are involved in behavioral phenomena [20]. The latter site of action of the vasopressinergic hormone can influence dissemination of tumor cells by luminal narrowing of lungs vessels under the effect of vasopressin.

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