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Does change in neurotransmitter brain status affect the growth of transplantable melanoma?

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ABSTRACT

Aim. To study the influence of the features of aminergic brain status on the development of B16/F10 melanoma in mice with urokinase gene knockout and chronic neurogenic pain (CNP).

Materials and methods. The study included female ($n = 68$) C57BL/6 mice with the normal urokinase gene (+*uPA*) and C57BL/6-PlautmI.IBug-This Plau6FDhu/GFDhu mice with urokinase gene knockout (–*uPA*). The model of CNP was created in the animals, and in 14 days B16/F10 melanoma was transplanted. The mice were euthanized 21 days after the transplantation. Levels of adrenaline (A), noradrenaline (NA), dopamine (DA), histamine (H), serotonin (5HT), 5-hydroxyindoleacetic acid (5HIAA) were determined in the brain using standard ELISA test systems (Cusabio, China).

Results. CNP in (+*uPA*) females resulted in the reduction of almost all studied biogenic amines (BA). On the opposite, (–*uPA*) females showed an increase in NA, DA, 5HT and a decrease of H. 5HIAA increased in both CNP and gene knockout. 5HT in (+*uPA*) females with CNP decreased, while its physiological level in gene knockout mice was maintained. After 3 weeks of tumor growth in animals with CNP, (+*uPA*) mice demonstrated increased levels of studied BA (except for 5HIAA) compared to mice with CNP alone. Only H increase was observed in (–*uPA*) mice from the similar group.

Conclusion. CNP in mice inhibited A-, NA-, H- and 5HT-ergic systems of the brain; the opposite effects were registered in urokinase gene knockout, except for the H-ergic system. Combination of CNP and melanoma in (+*uPA*) female mice activated all studied BA systems, and in (–*uPA*) females – H-ergic system only. Different stressful effects, CNP, and genetic disorders (urokinase gene knockout) contributed to changes in the brain BA system functions, leading to the activation of pro- or antitumor mechanisms.

Key words: B16/F10 melanoma, melanoma course, chronic neurogenic pain, *uPA* urokinase gene knockout, mice, brain, biogenic amines.

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Оказывает ли влияние изменение нейротрансмиттерного статуса мозга на рост перевивной меланомы?

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РЕЗЮМЕ

Цель. Изучить влияние особенностей аминергического статуса головного мозга у мышей при нокауте гена урокиназы и хронической нейрогенной боли (ХНБ) на развитие меланомы B16/F10.

Материалы и методы. Работа выполнена на самках мышей (n = 68) C57BL/6 – с полноценным геном урокиназы (+*uPA*) и C57BL/6-PlautmI.IBug-This Plau6FDhu/GFDhu – с нокаутом гена урокиназы (–*uPA*). Животным моделировали состояние ХНБ, через 14 сут подкожно перевивали меланому B16/F10. Забой производили через 21 сут после перевивки. В головном мозге определяли содержание адреналина (А), норадреналина (НА), дофамина (ДА), гистамина (Г), серотонина (5НТ), 5-оксииндолуксусной кислоты (5ОИУК) с помощью иммуноферментных стандартных тест-систем (Cusabio, Китай).

Результаты. У самок (+*uPA*) ХНБ приводила к снижению содержания практически всех исследованных биогенных аминов (БА). У самок (–*uPA*), напротив, отмечался рост концентрации НА, ДА, 5НТ и снижение Г. Обнаружено увеличение уровня 5ОИУК при ХНБ и нокауте. У самок (+*uPA*) с ХНБ снижался уровень 5НТ, но сохранялось его физиологическое содержание у мышей с нокаутом. Через 3 нед роста опухоли на фоне ХНБ у мышей (+*uPA*) обнаружено увеличение уровня всех изученных БА, кроме 5ОИУК, по сравнению с уровнем у мышей только с ХНБ. У мышей (–*uPA*) в аналогичной группе было увеличение только гистамина.

Заключение. ХНБ приводила к угнетению А-, НА-, Г-, 5НТ-ергических систем мозга мышей, а при нокауте гена урокиназы наблюдались противоположные эффекты, за исключением Г-ергической системы. Сочетание ХНБ и меланомы у самок мышей (+*uPA*) приводило к активации всех изученных систем БА, а у самок (–*uPA*) – только Г-ергической системы. Стрессорное воздействие – ХНБ, генетическое нарушение (нокаут гена урокиназы), способствовали изменению функционирования систем БА мозга, разнонаправленно влияя на противоопухолевые механизмы.

Ключевые слова: меланома, хроническая нейрогенная боль, нокаут гена урокиназы uPA, мыши, головной мозг, биогенные амины.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

The brain encloses a unique microenvironment which supports physiological homeostasis and responds to pathological changes, including cancer [1]. Skin cells that perceive both external and internal changes and participate in the regulation of the homeostasis in the body are able to influence systemic regulators, and particularly the neuroamine status of the brain [2]. The close connection between skin cells and brain neurons is confirmed by studies in some neurodegenerative diseases, in particular, Parkinson disease [3], and mental disorders which are often

combined with chronic skin diseases and melanoma [4, 5]. Multifunctional biogenic amines modulate the response of the central regulatory system to various internal and external impacts and participate in the body response to stress, playing a crucial role in maintaining homeostasis [6].

A number of studies have shown that stress can retard tumor growth through the action of neuroamines on the hypothalamus and immune system [7]. Scattered experimental data show the role of biogenic amines of the brain in antitumor protection [8, 9]. With acute and chronic stress, there is a change of the level of histamine in the brain, which regulates the secretion of hormones of the hypothalamus, the anterior pituitary gland, modulates the effect of certain transmitters [10], and plays an important role in multiple diseases of the central nervous system [11]. Comorbid diseases associated with tumor growth are connected with differences in treatment, clinical management, prognosis, survival, cancer progression, and can also cause a higher risk of complications and lower quality of life.

Moreover, despite the importance of taking comorbid diseases into account, there is not enough attention to the relation between melanoma and concomitant pathology [12]. While the effect of chronic neurogenic pain (CNP) on the transmitter status of the brain is beyond dispute [13, 14], the connection between the urokinase gene knockout and the balance of brain biogenic amines is not so obvious. There are experimental data on the significant role of plasminogen activators in the processes of the brain recovery after ischemic lesion [15]. Models of animals with neural lesion that causes chronic neuropathic pain and animals with a modified genotype allow studying multiple changes that occur in the main regulatory systems, as well as their possible impact on the tumor process development.

The aim of this work was to study the influence of characteristics of the aminergic status in the mice brain at the urokinase gene knockout and chronic neurogenic pain on the development of the B16/F10 melanoma.

MATERIALS AND METHODS

The work was performed on female mice ($n = 68$). All animals were kept under natural light conditions with free access to water and food. The studies were carried out according to the requirements and conditions set out in the International Guiding Principles for Biomedical Research Involving Animals and the Order of the Ministry of Health of the Russian Federation No. 267 of 19.06.03 "On the approval of laboratory practice rules". The mice of the C57BL/6 line with a full urokinase gene (+*uPA*), $n = 34$, were obtained from Scientific Center for Biomedical Tech- nologies of Andreevka of the Federal Biomedical Agency. Animals of the C57BL/6-PlautmI.IBug-This Plau6F-Dhu/GFDhu line with urokinase gene knockout (–*uPA*) (target mutation with production of a protein being unable to bind to the urokinase-type plasminogen activator receptor), $n = 34$, were obtained from the Laboratory Animal Breeding Station of "Pushchino".

The animals were divided into 8 groups: intact (+*uPA*) females ($n = 7$); (+*uPA*) females with CNP ($n = 9$); intact (–*uPA*) females ($n = 7$), (–*uPA*) females with CNP ($n = 9$); (+*uPA*) females with melanoma ($n = 9$); (+*uPA*) females with CNP and melanoma ($n = 9$); (–*uPA*) females with melanoma ($n = 9$); (–*uPA*) females with CNP and melanoma ($n = 9$).

The modeling of CNP by ligation of the sciatic nerve from both sides, the procedure for subcutaneous transplantation of the B16/F10 melanoma into the right subscapular region, the course of the experiment and the preparation of 10% of cytosolic fractions have been described before [13]. For this work, we used the B16/F10 murine melanoma obtained from the Research Center for Oncology named after N.N. Blokhin of the Ministry of Health of the Russian Federation. The tumor proliferative pool was 71.6%. The material for transplantation of the B16/F10 melanoma was obtained from (+*uPA*) donor mice of the C57BL/6 line, second passage, on days 12-16 of tumors development. The animals with tumors were euthanized 21 days after the melanoma transplantation. The 10% cytosolic fractions were obtained from the isolated brain and prepared on 0.1 M potassium phosphate buffer pH 7.4 containing 0.1% Tween-20 and 1% BSA; all manipulations were performed on ice. The content of biogenic amines — adrenaline (A), noradrenaline (NA), dopamine (DA), histamine (H), serotonin (5HT), 5-hydroxyindoleacetic acid (5HIAA), was determined using standard ELISA test systems (Cusabio, China).

The statistical processing of the material was carried out using the Statistica 10.0 software; mean values and standard errors ($M \pm m$) were determined. The significance of differences in average values was evaluated using the Mann – Whitney U-test and Student's t-test (after checking for normal distribution using the Shapiro – Wilk test). The differences were considered significant at $p < 0.05$.

RESULTS

First, there was a study of the effect of CNP, urokinase knockout and their combination on the content of biogenic amines in the female mice brain. The results of the study of biogenic amines in the brain of (+*uPA*) females with CNP showed a decrease in the levels of adrenaline by 2.4 times, of noradrenaline by 2.3 times, of histamine by 2.1 times and serotonin by 1.9 times ($p < 0.05$) against the background of an increase in 5HIAA by 3.3 times, compared with the indicators of intact (+*uPA*) females (Table 1). The dopamine content in the brain of (+*uPA*) females did not change under the influence of CNP, but there was an increase of the DA/NA ratio by 2.3 times and a decrease of the ($p < 0.05$) A/5HT ratio by 1.3 times as well as of the 5HT/5HIAA ratio by 4.8 times.

The urokinase gene knockout in female mice had no effect on adrenaline and serotonin levels in the brain, with an increase of the noradrenaline level by 3.2 times, dopamine level by 2.9 times, and 5HIAA level by 2.0 times, but the histamine content was decreased by 2.1 times (Table 1). As a result, the 5HT/5HIAA ratio coefficient decreased by 2.5 times, the ratio of DA/NA and A/5HT did not differ from indicators of intact (+*uPA*) females.

Modeling of CNP in (–*uPA*) females resulted in a decrease in the brain level of NA and DA by 1.5 times compared to (–*uPA*) females without pain ($p < 0.05$), however, relative to (+*uPA*) females, both intact and having the CNP background, the concentration of these neurotransmitters was higher: NA by 1.7 times and 4 times, respectively, and DA by 2 times ($p < 0.05$).

In (–*uPA*) females, CNP caused a decrease in histamine by 1.8 times compared with intact (–*uPA*) and (+*uPA*) animals ($p < 0.05$), without statistically significant differences compared with (+*uPA*) females with CNP. At the same time, there was an increase in serotonin content by an average of 3 times compared with intact (–*uPA*) and (+*uPA*) animals and 5.2 times compared with (+*uPA*) females with CNP ($p < 0.05$). Modeling of CNP in (–*uPA*) mice resulted in an increase of the brain concentration of 5HIAA by 1.7 times and 1.3 times ($p < 0.05$) compared with (–*uPA*) and (+*uPA*) intact animals, respectively, while there were no significant differences compared to (+*uPA*) females with CNP. As a result, the A/5HT ratio in the brain decreased by more than 3.4 times and 2.8 times compared to intact (–*uPA*) and (+*uPA*) females and 2.1 times compared to (+*uPA*) females with CNP, and the ratio between serotonin and its 5HIAA metabolite did not differ from (+*uPA*) intact animals, but was 2.2 times higher than the one of (–*uPA*) intact animals and 4 times higher than the one of (+*uPA*) females with CNP. The DA/NA coefficient in the brain of (–*uPA*) females with CNP did not have significant differences from (–*uPA*) and (+*uPA*) of intact animals, but it turned out to be 2 times lower than that of (+*uPA*) females with CNP.

The urokinase gene knockout and CNP contributed to a change in the biogenic status of the brain in different directions. If in (+*uPA*) females, CNP caused a decrease in the A and NA level, then the urokinase gene knockout, on the contrary, resulted in an increase in the NA and DA content, but did not affect the A value. The development of CNP in (–*uPA*) females was accompanied by a decrease not only in the brain level of NA, like in (+*uPA*) females with the CNP background, but also in the level of DA; however, the levels of these catecholamines remained significantly higher than in females with a normal genome, both with and without CNP.

Only in (–*uPA*) females, the A/5HT ratio in the brain was higher than the one in (+*uPA*) females; CNP reduced the A/5HT ratio both independently and in combination with knockout. It should be noted that in females, any of the exerted effects – CNP, urokinase gene knockout, alone or in combination with CNP, resulted in an increase in the brain level of 5HIAA, a serotonin metabolite, as well as in a decrease in the histamine concentration.

Previously, we found that CNP in mice with a normal genome had a stimulating effect on the development of transplanted melanoma, which was expressed in a decrease in life expectancy and latent period, as well as in more active metastatic spreading to non-specific sites against the background of a smaller tumor volume [13]. The development of transplanted melanoma in mice with the *uPA* gene knockout was characterized by smaller tumor volumes and the absence of metastatic spreading after 3 weeks, against the background of the absence of differences in life expectancy and latent period. In female mice with the urokinase gene knockout and CNP with transplanted melanoma, a shorter life expectancy was observed, which is characteristic of animals with CNP and tumor growth, however, there was a larger latent period [16].

Taking into account the multidirectional changes in the neurotransmitter status of the brain in female mice under the influence of CNP and the urokinase gene knockout, as well as differences in the course

of malignant process, the content of biogenic amines in the brain was studied in female mice with CNP, the urokinase gene knockout and their combination after 3 weeks of growth of transplanted melanoma B16/F10.

As a control, the level of biogenic amines in (+*uPA*) female mice with self-developing melanoma (without concomitant CNP) was examined first. After 3 weeks of growth of the B16/F10 melanoma, there was an increase of the brain level of dopamine by 1.8 times ($p < 0.05$), of serotonin by 2.3 times and of 5HIAA by 1.5 times ($p < 0.05$); the content of noradrenaline, histamine and adrenaline did not have significant differences from values in intact (+*uPA*) females (Table 1). As a result, the A/5HT ratio decreased by 2.1 times, while the 5HT/5HIAA and DA/NA ratios increased by 1.5 times ($p < 0.05$).

In (+*uPA*) females with CNP, after 3 weeks of growth of melanoma in the brain, there was an increase of levels of adrenaline and dopamine by 1.4 times ($p < 0.05$), of noradrenaline by 2.2 times, of histamine by 2.0 times and of serotonin by 1.3 times ($p < 0.05$), together with a decrease in the content of 5HIAA by 2.5 times, compared with indicators in the brain of (+*uPA*) females with CNP only (Table 1). As a result, the A/5HT ratio did not differ from the values in the brain of (+*uPA*) females with CNP only, 5HT/5HIAA was 3.3 times higher, and DA/NA, on the contrary, 1.6 times ($p < 0.05$) lower. It should be noted that compared with the amine content in animals with standard transplantation of melanoma, differences were also detected: lower levels of adrenaline, serotonin and the 5HT/5HIAA ratio by 1.8 times ($p < 0.05$), 2.4 times and 2.1 times, respectively (Table 1).

Table 1

Content of biogenic amines in the brain of female C57Bl/6 mice, $M \pm m$

Parameter	Intact (+*uPA*) mice	(+*uPA*) mice + melanoma B16/F10	(+*uPA*) mice + CNP	(+*uPA*) mice + CNP + melanoma B16/F10	Intact (–*uPA*) mice	(–*uPA*) mice + melanoma B16/F10	(–*uPA*) mice + CNP	(–*uPA*) mice + CNP + melanoma B16/F10
Adrenalin, ng/g of tis.	8.5 ± 0.7	9.0 ± 0.8	3.5 ± 0.3[1]	5.0 ± 0.6[1.3.4]	8.6 ± 0.85	7.4 ± 0.69[4]	8.6 ± 0.82[3]	8.1 ± 0.78
Noradrenaline, ng/g of tis.	19.4 ± 1.8	23.2 ± 2.2	8.5 ± 1.1[1]	18.9 ± 2.0[3]	61.4 ± 5.9[1]	40.3 ± 0.38[2.4]	33.7 ± 3.1[2.3]	37.0 ± 3.2
Dopamine, ng/g of tis.	17.5 ± 1.8	31.0 ± 3.1[1]	17.9 ± 1.5	25.0 ± 2.2[1.3]	50.0 ± 4.7[1]	33.4 ± 0.31[2]	35.8 ± 3.4[2.3]	41.2 ± 3.9
Histamine, ng/g of tis.	34.9 ± 3.2	30.4 ± 2.9	16.6 ± 1.2[1]	33.5 ± 2.9[3]	16.4 ± 1.5[1]	34.0 ± 0.32[2]	18.9 ± 1.6	28.2 ± 2.7[5]
Serotonin, ng/g of tis.	0.43 ± 0.03	0.97 ± 0.05[1]	0.23 ± 0.02[1]	0.4 ± 0.03[3.4]	0.35 ± 0.03	1.9 ± 0.15[2.4]	1.2 ± 0.11[2.3]	1.2 ± 0.10[6]
5-HIAA, µg/g of tis.	0.15 ± 0.03	0.23 ± 0.03[1]	0.5 ± 0.03[1]	0.2 ± 0.01[1.3]	0.3 ± 0.03[1]	0.4 ± 0.03[2.4]	0.5 ± 0.04[2]	0.4 ± 0.03
A/5HT	19.77 ± 1.4	9.28 ± 0.73[1]	15.22 ± 1.2[1]	12.5 ± 1.2[1.4]	26.1 ± 0.24[1]	3.89 ± 0.35[2.4]	7.17 ± 0.69[2.3]	6.75 ± 0.66[6]
5HT/5HIAA	2.9 ± 0.25	4.22 ± 0.39[1]	0.6 ± 0.05[1]	2.0 ± 0.18[1.3.4]	1.1 ± 0.09[1]	4.75 ± 0.47[2]	2.4 ± 0.18[2.3]	3.0 ± 0.03[6]
DA/NA	0.9 ± 0.08	1.34 ± 0.12[1]	2.1 ± 0.20[1]	1.32 ± 0.13[1.3]	0.81 ± 0.07	0.83 ± 0.08	1.06 ± 0.09[3]	1.11 ± 0.1

Note. [1] – statistically significant compared with intact (+*uPA*) animals; [2] – statistically significant compared with intact (–*uPA*) animals; [3] – statistically significant compared with (+*uPA*) animals with CNP; [4] – statistically significant compared with (+*uPA*) animals with the B16/F10 melanoma; [5] – statistically significant compared with (–*uPA*) animals with CNP; 6 – statistically significant compared with (–*uPA*) animals with the B16/F10 melanoma.

The level of catecholamines in the brain of (–*uPA*) mice decreased 3 weeks after tumor transplantation: adrenaline by 1.2 times ($p < 0.05$), noradrenaline and dopamine by 1.5 times ($p < 0.05$), and histamine, serotonin and 5HIAA, on the contrary, increased by 2.1 times, 5.8 times and 1.3 times ($p < 0.05$), respectively, compared with (–*uPA*) females. Moreover, the A/5HT ratio turned out to be 6.7 times lower, and the 5HT/5HIAA ratio was 4.3 times higher. It should be noted that, compared with the indicators of (–*uPA*) females with melanoma without concomitant CNP, the content of NA in (–*uPA*) mice was higher by 1.7 times ($p < 0.05$), of serotonin by 2.0 times, of 5HIAA – by 1.7 times ($p < 0.05$), but there was a decrease of the ratio of A/5HT by 2.4 times and of DA/NA by 1.6 times ($p < 0.05$).

The melanoma growth in female mice with the urokinase gene knockout and CNP was characterized by a low life expectancy 1.6 times ($p < 0.05$) lower than for females with the urokinase gene knockout without CNP, but with a latent period being larger by 1.7 times ($p < 0.05$). At that, in the brain of (–*uPA*) females with

CNP after 3 weeks of growth of melanoma, only a 1.5-fold increase ($p < 0.05$) in histamine levels was detected compared with the brain indicators of (*–uPA*) females with CNP without tumor transplantation (Table 1). The main difference compared with the neurotransmitter status in (*–uPA*) females with melanoma after 3 weeks of tumor growth was the decrease in the serotonin level by 1.6 times ($p < 0.05$), resulting in an increase in the A/5HT ratio by 1.7 times ($p < 0.05$) and a decrease in the 5HT/5HIAA ratio by 1.6 times ($p < 0.05$).

DISCUSSION

The development of transplanted melanoma is accompanied by a violation of central regulatory mechanisms, while the characteristics of the tumor development may depend on the initial status and reactivity of the aminergic systems of the brain. In the course of this study, it was found that the growth of melanoma at standard transplantation to female mice, after 3 weeks of the experiment, resulted in an imbalance of the dopaminergic and noradrenergic systems, with the prevalence of the first one, as well as in the serotonergic system activation, which was expressed in an increase of levels of both serotonin and its metabolite.

First of all, it is noteworthy that CNP (in (*+uPA*) females) and the urokinase gene knockout in independent variants have different effects on the adrenergic, noradrenergic and serotonergic systems, but these are equal in relation to the histaminergic system. From the obtained results, it can be seen that CNP in (*+uPA*) females results in a decrease of almost all of the studied biogenic amines in the brain, resulting in a violation of basic mechanisms of central regulation. The urokinase gene knockout, on the contrary, activates the noradrenergic, serotonergic and dopaminergic systems in the brain of females, as well as changes the biogenic amines ratio.

The model of chronic neurogenic pain is a model of chronic prolonged stress, while animals with the urokinase knockout, although having a genetically determined disorder, do not phenotypically differ from (*+uPA*) mice of the C57BL/6 line [15]. It is known that urokinase and its receptor are found in large quantity in the developing brain [17], but their expression in the adult state is limited to certain groups of neurons, mainly in the hippocampus and some subcortical structures [18]. In our study, it was shown that the urokinase gene knockout, although it did not have phenotypic manifestations in the behavior of mice, was characterized by a significant increase in the brain levels of noradrenaline, dopamine, and serotonin, but a decrease in histamine. We suppose that the found increase in the absolute levels of dopamine, noradrenaline and serotonin, as well as a change in the balance of biogenic amines in the brain of females with the urokinase gene knockout, is one of the mechanisms that contribute to the restoration of neurogenesis and synaptic transmission of nerve endings in case of various stress impacts under conditions of genetic damage to one of the links of the urokinase system.

The growth of most types of malignant tumors depends on the balance between factors promoting proliferation, angiogenesis, migration, and survival of the cells, and those that are involved in cell differentiation, inhibit proliferation and result in apoptosis [19]. Therefore, after transplantation of malignant melanoma cells into animals with different neurotransmitter status of the brain, which was genetically determined or altered as a result of prolonged chronic exposure of neurogenic pain, the development of the malignant process may turn out to be modified due to various background states of central regulatory systems.

The decrease in biogenic amines in the brain of females under the influence of CNP resulted in a weakening of the antitumor defense of the body, which resulted in a decrease in life expectancy, latent period, and increase in activity and metastasis sites in animals. At the same time, the growth of transplanted melanoma in female mice with the urokinase gene knockout did not have significant differences in life expectancy and latent period; however, the increase in tumor volume was significantly slower than at transplanting melanoma to mice with a wild type of the urokinase gene. We suggest that the urokinase gene knockout could have an antitumor effect at the local level, as we previously showed the participation of the system of growth factors and fibrinolysis in the pathogenesis of melanoma growth in female mice [20]. Experimental studies show the interaction of serotonergic and dopaminergic systems with each other, while affecting the brain plasticity and the ability of the body as a whole to recover and adapt [21]. Noradrenaline is involved in the protection of dopaminergic neurons through enhancing the tyrosine hydroxylase expression [22]. The depletion of the noradrenergic system of the brain is often associated with hyperactivation of the hypothalamo-pituitary-adrenal axis, which characterizes various mental disorders associated with stress, such as anxiety and depression, and neurodegenerative conditions, such as Alzheimer disease and multiple sclerosis [23]. Recent studies show that this reaction has

gender differences, and females are less resistant to prolonged stress [24].

In this study, animals with CNP and the urokinase gene knockout showed a decrease in histamine levels in the brain. This fact, on the one hand, can be explained by the multifunctionality of histamine, which takes part both in nociceptive reactions and in restoration processes in the brain. The difficulty in interpreting the results of changes in neuronal amines in general and histamine in particular lies in the activation of various receptors, which results in various effects. On the other hand, an increase in aminoxidase activity may cause a decrease in histamine levels. We have detected an increase in the level of the serotonin metabolite – 5HIAA both in CNP and knockout animals. A significant difference was a decrease in serotonin in females with CNP, but the preservation of its physiological concentrations in knockout animals.

Moreover, the combined effect of CNP and (*–uPA*) results only in a decrease in the effect of the urokinase gene knockout and activation of the serotonergic system metabolism against the background of an increase in the serotonin level. There is a possibility that it was the increase in the serotonin level in the brain of females with the combination of the gene knockout with CNP that affected the increase in the latent period of tumor development, although it did not result in an increase in the life expectancy of animals.

CONCLUSION

Thus, the obtained study results indicate the undoubted effect of changes in the neurotransmitter balance of the brain under the influence of chronic neurogenic pain and genetically determined urokinase deficiency on the growth of transplanted melanoma. A better understanding of mechanisms underlying neurotransmitter function at oncogenesis, neurogenesis, and chronic comorbid diseases will make it possible to predict the possible course of the disease and to develop personalized antitumor therapy.

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Frantsiyants E.M., Kaplieva I.V. – conception and design of the experiment. Frantsyants E.M., Kaplieva I.V., Bandovkina V.A. – analysis and interpretation of results. Bandovkina V.A., Surikova E.I. – drafting and editing of the manuscript, critical revision for important intellectual content. Trepitaki L.K. – carrying out of the experiment. Cheryarina N.D. – carrying out of ELISA analysis. Kit O.I., Frantsyants E.M., Kotieva I.M. – final approval of the manuscript for publication.

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