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# Chronic neurogenic pain is responsible for changes in concentrations of biogenic amines in the brain in urokinase knockout mice with melanoma B16/F10

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## Abstract

Our aim is to study special features of actions and effects of chronic neurogenic pain on concentrations of biogenic amines in the brain in tumor-bearing urokinase knockout mice (uPA).

## Materials and methods

Our research work has been conducted in female/male mice line C57BL/6 (uPA+/+) n=48 and line C57BL/6-Plautm1.1Bug-This-PlauGFDhu/GFDhu (uPA) -/-) n = 48 with targeted mutation of protein incapable of binding to receptor of the urokinase-type plasminogen activator, i.e. in the urokinase knockout (uPA) mice. The animals of each line have been divided into subgroups (n= 6) as follows: the intact animals, the chronic neurogenic pain mice (CNP), the mice with the growth of inoculated melanoma (B16/F10) and the mice with combination melanoma growth plus chronic neurogenic pain (B16/F10 + CNP). In 10% of the brain homogenates, with the use of standard analysis system method ELISA (Cusabio. China), we have determined concentrations of adrenaline (A), noradrenaline (NA), dopamine (DA), histamine, serotonin (5HT) and 5-hydroxyindoleacetic acid (5-HIAA). Obtained statistic data have been processed using the Statistica 10.0 package.

## Results

The concentration of biogenic amines in the brain in the intact uPA-/- mice has been found to be higher than in the intact uPA+/+ animals, and the response to the growth of inoculated melanoma B16/F10 and CNP under single-factor action and combined-action variants depends both on the sex and the condition of the urokinase gene. In the uPA-/- female/male mice in response to the only CNP and the only B16/F10 growth the serotonergic system in the brain has been activated. In contrast thereto, in case of the melanoma growth against the CNP background, we have revealed a decrease in the level of 5HT, DA in the female/male mice and NA in the female animals.

## Conclusion

One of the factors resulting in cancellation of the genetically determined inhibition of the growth of inoculated melanoma B16/F10 under its combination with developing CNP in the uPA-/- mice is suppression of their noradrenergic, serotonergic and dopaminergic systems, initially activated in the intact uPA-/- animals to compensate their urokinase deficit.

## Keywords

C57/B16 mice line, UPA, Brain biogenic amines, Chronic neurogenic pain, Inoculated melanoma B16/F10

## Imprint

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## Introduction

The necessity of the synaptic plasticity in the process of neurorepair in the brain under different pathology conditions have been recognized by a number of researchers [1,2,3]. An important role played by the brain urokinase system in neurogenesis, migration and growth of neurons, synaptic plasticity [4] and neuroprotection [1,2,3], that is to say, in the mechanisms having pathogenetic significance and relevance under different traumas, stressor actions as well as neurodegenerative and psychological diseases like Alzheimer's disease, Parkinson's disease, autism, epilepsy, schizophrenia and depression [5,6]. The urokinase knockout animals show some abnormalities related to disorders in regeneration, have greater susceptibility to infection agents and lowered metastatic activity of malignant

tumors. However in the mice demonstrating an uPA deficit, as opposed to the uPAR knockout animals, we do not observe an increased susceptibility to spasms and disorders in fine motor activities and muscle coordination [7]. Probably it might be connected with a possibility of substitution of uPA in the brain for other ligands for uPAR like vitronectin, kininogen or SRPX2 as well by a number of neuromediators [6,7].

Some researchers strengthen the role of biogenic amines: serotonin, noradrenaline, dopamine in the brain mechanism of plasticity under various stressor actions and effects including chronic neurogenic pain, trauma conditions, neurodegenerative diseases, ageing [8]; it is likely that malignant progression can be classified as a stress-type action exerted on an organism.

The neuropathic pain is correlated to depression, Parkinson's disease and anxiety that is evidenced by the action of pain inhibiting antidepressants [9,10]. Neuroplastic and glial changes in the brain produced in experiments with CNP demonstrate in CNS an alteration of expression of peptide receptors, neuroimmune and neuromediator factors as well as disorders in activity of the ion channels in different structures in the brain that leads to a disorder in the regulation of the noradrenaline control of pain [11]. Besides, using the model of chronic visceral pain in rats, it has been discovered that the dopamine system is involved in the regulation of corticotropin-releasing hormone in the brain in the paraventricular nucleus that bears witness to the fact that there is a change in the functional activity of the major regulator axes in the organism, in particular of the hypothalamic-hypophyseal-adrenal axis [12].

The dopamine dysfunction is connected with an increased sensitivity to pain under some chronic pain conditions including headache, fibromyalgia and osteoarthritis, and Parkinson's disease, representing the classical hypodopaminergic state, is associated with a rise in occurrence rate of depressions and chronic pain [13]. In this case, serotonin is widely projected in the brain to modulate synapses, it regulates release and functioning of noradrenaline and dopamine, participating at the same time in the modulation of pain, that is to say, that transmission of signals 5-HT can reveal both antidepressant and antinociceptive properties with modulating the synaptic connection and transmission of other monoamine signals in various regions in the brain [13].

One more interesting multifunctional biogenic amine is histamine. Histamine being a mediator is synthesized in histaminergic neurons, which are located in the posterior hypothalamus, and it spreads throughout the entire organism [14]. There are intense studies on the role of histamine in pathogenesis of different neurological and psychiatric disorders [15]. It has been confirmed that degranulation of mast cells activates the nociceptors of meningeal cells, and in case of histamine introduction into cerebrospinal fluid or brain structures histamine reduces the nociceptive transmission [16]. In some researches supported is a concept that there is a close relationship between histamine and other neuromediators, where the modulation of noradrenergic, dopaminergic and serotonergic pathways is provided [17, 18].

## Aim

The aim of these studies is to investigate peculiarities of actions and effects produced by chronic neurogenic pain on concentrations of biogenic amines in the brain in tumor-bearing urokinase knockout (uPA) mice.

## Materials and methods

Our studies have been carried out in 96 female/male mice. The animals of line C57BL/6 (uPA+/+) n=48 have been delivered to us by the Federal State Medical & Biological Institution "Research Center of Biomedical Technologies at the Federal Medical & Biological Agency", Branch Andreevka. The mice of line mC57BL/6-Plautm1.1Bug-ThisPlauGFDhu/GF-Dhu (uPA) -/-) n = 48 with an initial individual body mass of 26–31 g have been received from the Scientific Production Enterprise "Laboratory Animals' Breeding Facility" at the Branch of the Institution of Bioorganic Chemistry named after Academy Members M.M.Shemyakin and Yu.A.Ovchinnikov (Pushchino); in the animals of this line produced gave been targeted mutations of protein, which is incapable of binding to receptor of urokinase-type plasminogen activator, i.e. with the urokinase knockout (uPA).

The tested animals have been kept under natural light conditions with a free access to water and food. All research works have been conducted in full conformity with applicable requirements and conditions stated in "International Recommendations on Pursuance of Medical & Biological Research in Animals" and Order No.267 "Approval of Regulations on Laboratory Practice" dd. 19.06.03 issued by the Ministry of Health Care of the Russian Federation.

The uPA-/- and uPA+/+ animals have been divided in the following subgroups (n= 6): intact mice; mice with chronic neurogenic pain (CNP mice) caused by bilateral sciatic nerve ligation [19]; mice showing growth of inoculated melanoma (B16/F10) and mice with a combination of melanoma and CNP (B16/F10+CNP).

In our studies, we have used mice melanoma line B16/F10 delivered by FSBI "National Medical Research Center for Oncology named after N.N.Blokhin" at the Ministry of Health Care of Russia. Immediately after decapitation, the animals' brain has been removed for further analysis, the 10% homogenate of the white matter on ice in 0,1 M potassium phosphate buffer, pH 7.4, containing 0,1% Tween-20 and 1% BCA, has been prepared, where we according to the standard test system with the ELISA (Cusabio, China) method have analyzed the prepared samples for concentrations of adrenaline (A), noradrenaline (NA), dopamine (DA), histamine, serotonin (5HT) and 5-hydroxyindoleacetic acid (5-HIAA).

Obtained statistic data have been processed using the Statistica 10.0 package. The Shapiro-Wilk criterion test has been used to test for fit to the normal distribution. The Mann-Whitney test is employed to evaluate the significance of variances between samples that has been taken to be $p<0.05$. The data are presented as an average value ± standard error of the average.

## Results

It has been found (see Table 1 herein) that in the brain in the intact uPA-/- male mice the concentration of noradrenaline is 3,5 times higher than it is found in the intact uPA+/+ males; the dopamine level has been recorded to be 2,1 times higher against the intact uPA+/+ males, and the serotonin concentration has been identified 1,9 times greater as compared with the above intact mice cohort; at the same time, the 5-HIAA concentration has been found 1,8 times lower and the histamine level has been reported to be 2,2 lower against the respective intact cohort. In this case, the adrenaline concentration in the brain in the urokinase knockout mice has shown no difference with that found in the uPA+/+ animals.

CNP has produced its effect on concentrations of the tested amines in the brain in the uPA-/- males with decreasing the noradrenaline level by 1,8 times, but with activating at the same time the serotonergic system, with increasing the 5HT content by 1,5 times against the background of a rise in the 5-HIAA concentration by 1,4 times ($p<0,05$). In the uPA+/+ males the change in the concentrations of biogenic amines in the brain has been found to be not so pronounced, and it has affected the adrenaline level only, which has been determined to be 1,6 lower than that recorded in the intact animals.

The growth of inoculated melanoma B16/F10, similar to the case with CNP, in the uPA-/- males has resulted in activation of their serotonergic system with increasing the concentrations of 5HT and 5-HIAA by 2,5 and 1,8 times, respectively, as compared with the intact animals. Under growing melanoma B16/F10, the levels of adrenaline and histamine in the brain in the uPA-/- males have been reported to be 1,3 and 1,7 times greater against a reduction in the dopamine level by 1,6 times ($p<0,05$), while the noradrenaline concentration has remained unchanged, as compared with the intact uPA-/- males.

In contrast to the above tendencies, the growth of melanoma in the uPA+/+ males has induced inhibition of the serotonergic system that has been reported as a decrease in the 5HT level by 1,5 times and a reduction in the 5-HIAA concentration by 2,6 times; moreover the dopamine concentration value in the brain has been found to be 8,2 times lower than it is identified in the intact uPA+/+ males, and recorded has been an increase in the adrenaline content by 4,1 times and a rise in the noradrenaline concentration by 2,1 times ($p<0,05$).

The growth of melanoma against the CNP background in the uPA-/- males have made its effect on the concentrations practically of all investigated BA, with the exception of adrenaline, that has resulted, in comparison with the initial indicators (the CNP reference), in a rise of the noradrenaline content by 1,5 times in the brain, an increase in the histamine level by 1,4 times, a growth of the 5-HIAA content by 1,4 times, while the 5HT content has been decreased by 5,3 times and the dopamine concentration has been reduced by 1,4 times ($p<0,05$). As a consequence, it has been found that in the uPA-/- males the noradrenaline concentration has become 1,3 times lower and the serotonin content has decreased by 8,6 times as compared with the respective data in the animals having the growing melanoma only, referred to the equivalent period of time. In the brain in the uPA+/+ males with the melanoma growth against the back-

Table 1
Concentrations of biogenic amines in the brain in male mice C57Bl/6 (uPA+/+) and C57BL/6-Plautm1.1Bug-ThisPlauGFDhu/GFDhu (uPA-/-)

| Groups | | Adrenaline (ng/g tissue) | Noradrenaline (ng/g tissue) | Dopamine (ng/g tissue) | Histamine (ng/g tissue) | Serotonin (ng/g tissue) | 5-HIAA 5 (mg/g tissue) |
|---|---|---|---|---|---|---|---|
| C57Bl/6 (uPA+/+) | Intact | 5,1±0,5 | 17,9±1,9 | 32,7±2,8 | 27,6±2,3 | 0,62±0,05 | 0,45±0,04 |
| | Reference (CNP) | 3,1±0,3[1] | 17,0±1,5 | 33,8±3,2 | 27,5±2,5 | 0,65±0,06 | 0,42±0,039 |
| | B16/F10 | 21,1±1,9[1] | 37,2±3,3[1] | 4,0±0,39[1] | 25,4±2,3 | 0,42±0,04[1] | 0,17±0,016[1] |
| | B16F10+CNP | 4,5±0,4[2,3] | 20,7±1,8[3] | 26,6±2,5[2,3] | 16,5±1,4[2,3] | 0,33±0,02[2,3] | 0,35±0,03[3] |
| C57Bl/6 (uPA-/-) | Intact | 5,7±0,3 | 62,3±5,9[4] | 69,0±6,7[4] | 12,5±1,3[4] | 1,2±0,3[4] | 0,25±0,02[4] |
| | Reference (CNP) | 5,9±0,5 | 34,3±2,4[1] | 77,4±7,2 | 15,3±1,4 | 1,85±0,15[1] | 0,34±0,03[1] |
| | B16/F10 | 7,3±0,6[1] | 65,2±5,8 | 44,5±4,3[1] | 21,2±2,0[1] | 3,0±0,29[1] | 0,46±0,03[1] |
| | B16F10+CNP | 7,0±0,7 | 51,1±4,9[2,3] | 54,6±5,1[2] | 20,8±1,7[2] | 0,35±0,03[2,3] | 0,47±0,04[2] |

Notes: statistically significant [1] – as compared with intact animals; [2] – as compared with the reference (CNP); [3]- as compared with the only melanoma growth condition; [4]- as compared with the respective group in mice uPA+/+ (p< 0,05)

ground of CNP, as compared with the indicators of the respective reference cohort (CNP), the level of adrenaline has increased by 1,5 times, but at the same time the concentration of dopamine has become 1,3 lower, the content of histamine has decreased by 1,7 times and that of serotonin has been reduced by 2 times, while the 5-HIAA concentration has remained unchanged. As a result, in comparison with the mice having the melanoma B16/F10 growth only, in the brain in the uPA+/+ males we have found that the adrenaline concentration has become 4,7 times lower, the noradrenaline level has been reduced by 1,8 times, the histamine content has decreased by 1,5 times, and the serotonin concentration has become lower by 1,3 times, but at the same time, the concentration of dopamine has become 6,7 times greater and that of 5-HIAA has been recorded to be 2,1 times higher, respectively (p<0,05).

As to the brain in the intact uPA-/- females, when comparing them with the uPA+/+ female mice (see Table 2 herein), the following data changes have been detected: the noradrenaline content is 3,2 times higher, the concentration of dopamine is 2,9 times greater and that of 5-HIAA has become 2,1 times higher, however at the same time reported has been a reduction in the concentration of histamine by 2,1 times and in the content of serotonin by 1,3 times, respectively (p<0,05).

It has been detected that CNP has produced in the brain in the uPA-/- females an effect of increase in the serotonin concentration by 4,5 times and a rise in the 5-HIAA content by 1,6 times, while the noradrenaline concentration and the dopamine content have been found by 1,8 and 1,4 times lower, respectively, (p<0,05), as against the intact uPA-/- females. At the same time, we have found that CNP has induced in the brain a decrease in the adrenaline content by 2,3 times, the noradrenaline concentration by 2,2 times, the histamine content by 2,1 times and the serotonin concentration by 1,8 times, against the background of an increase in the 5-HIAA content by 2,3 times, but with no change in the dopamine concentration.

The progression of melanoma B16/F10 in the uPA-/- females has resulted in a reduction in the concentration of noradrenaline and that of dopamine by 1,5 times, but at the same time it has induced an increase in the histamine content by 2,1 times, in the serotonin level by 2,2 times against the background of a rise in the 5-HIAA content by 1,3 times, as compared with the respective data in the intact uPA-/- females. As to the uPA+/+ female mice, the growth of inoculated melanoma B16/F10 has produced an effect of an increase in the dopamine level by 1,8 times, in the serotonin concentration by 2,3 times and in the 5-HIAA content by 1,5 times (p<0,05) as against the intact mice cohort, with no change in the adrenaline, noradrenaline and histamine levels.

The combination of CNP and the melanoma B16/F10 growth in the uPA-/-females has initiated a decline in the level of noradrenaline, dopamine and serotonin by 1,4, 1,7 and 2,8 times, respectively, but at the same time recorded has been a rise in the histamine concentration by 1,5 times (p<0,05) as compared with the initial background indicators in the reference CNP cohort. As against the respective data in the uPA-/- females, having growing melanoma only, those uPA-/- female mice, who have been affected by the combined unfavorable factors (B16/f10 + CNP), have demonstrated a decrease in the level of noradrenaline by 1,7 times, in the concentration of dopamine by 1,6 times and in the content of serotonin by 1,4 times (p<0,05).

In case with the uPA+/+ females under the B16/F10 melanoma progression against the background

Table 2
Concentrations of biogenic amines in the brain in female mice line C57Bl/6 (uPA+/+) and C57BL/6-Plautm1.1Bug-ThisPlauGFDhu/GFDhu (uPA-/-)

| Groups | | Adrenaline (ng/g tissue) | Noradrenaline (ng/g tissue) | Dopamine (ng/g tissue) | Histamine (ng/g tissue) | Serotonin (ng/g tissue) | 5-HIAA (mg/g tissue) |
|---|---|---|---|---|---|---|---|
| C57Bl/6 (uPA+/+) | Intact | 8,5±0,7 | 19,1±1,9 | 17,5±1,7 | 34,9±3,2 | 0,43±0,04 | 0,15±0,01 |
| | Reference (CNP) | 3,7±0,36[1] | 8,5±0,8[1] | 17,9±1,6 | 16,6±1,5[1] | 0,24±0,02[1] | 0,48±0,04[1] |
| | B16/F10 | 9,0±0,8 | 23,2±2,3 | 31,0±3,0[1] | 30,4±2,9 | 0,97±0,05[1] | 0,23±0,02[1] |
| | B16F10+CNP | 5,1±0,42,3 | 19,0±1,8[2] | 27,6±2,5[2] | 33,6±2,5[2] | 0,45±0,02[2,3] | 0,21±0,01[2] |
| C57Bl/6 (uPA-/-) | Intact | 8,5±0,79 | 61,4±5,9[4] | 50,0±4,7[4] | 16,4±1,5[4] | 0,33±0,03[4] | 0,32±0,02[4] |
| | Reference(CNP) | 8,6±0,81 | 33,7±3,1[1] | 35,8±3,2[1] | 18,9±1,7 | 1,5±0,18[1] | 0,50±0,04[1] |
| | B16/F10 | 7,4±0,6 | 40,3±4,8[1] | 33,4±3,31 | 33,9±3,1[1] | 0,74±0,06[1] | 0,43±0,038[1] |
| | B16F10+CNP | 8,1±0,78 | 23,6±2,2[2,3] | 21,0±1,8[2,3] | 28,2±2,1[2] | 0,54±0,05[2,3] | 0,43±0,04 |

Notes: statistically significant [1] – as compared with intact animals; [2] – as compared with the reference (CNP); [3]- as compared with the only melanoma growth condition; [4]- as compared with the respective group in mice uPA+/+ (p< 0,05)

of CNP, as compared with the initial background (the reference: CNP), we have recorded in the brain an increase in the concentrations of adrenaline by 1,4 times, noradrenaline by 2,2 times, dopamine by 1,5 times, histamine by 2 times, serotonin by 1,9 times against the background of lowering of the 5-HIAA content by 2,3 times (p<0,05). In this case, as compared with the respective indicators in the uPA+/+ females with the growing B16/F10 melanoma only, the females with combination melanoma + CNP have demonstrated a decline in the levels of adrenaline and serotonin by 1,8 and 2,2 times, respectively (p<0,05).

## Discussion

As it has been evidenced above, we have shown an inhibition of the inoculated melanoma progression in the urokinase knockout mice, particularly in the uPA-/- as compared with the uPA+/+ animals [20]. At the same time, CNP has restricted initiating of stress-limiting mechanisms in the brain in the uPA+/+ mice that has resulted in a more aggressive course of the malignant process and cancelled the genetically determined inhibition of the growth of the tumor in the uPA-/- mice cohort [20,21].

In the intact female/male uPA-/- animals in their brain we have detected a higher concentration of noradrenaline and dopamine, while the level of histamine has been recorded lower than it is recorded in the intact uPA+/+ mice. Research papers stating interactions between urokinase and biogenic amines, in particular, with the dopaminergic system of the brain, are few in number [22]. Some researches bear witness to the activation of the uPA and uPAR urokinase system as well as some neurotransmitters as a response to ischemic damage of the brain [3, 23]. So, we may suggest that the genetically determined deficiency in urokinase in the organism in the uPA-/- mice can be compensated to a certain degree by changes in the neuromediator's background of the brain. As to the uPA-/- animals, we have noted that there are some sex-related differences found in the performance of their serotonergic system. So, in the uPA-/- females we have identified an increase in the 5-HIAA content against the background of a decrease in 5HT, while in the males, in contrast thereto, we have detected a decline in the 5-HIAA content and an increase in the 5HT as compared with the intact uPA+/+ animals. Probably the uPA-/- females demonstrate a higher activity of deamination ferments 5HT (MAO) as compared with the uPA+/+ animals that is evidenced by an increased level of metabolite 5-HIAA against the background of a decrease in the 5HT substrate content. As opposed thereto, in the uPA-/- males the activity of the deamination ferments - monoamines might be inhibited, since the level of metabolite 5-HIAA has been found to be elevated, while the level of substrate 5HT has been recorded to be increased in comparison with the uPA+/+ males.

It should be pointed out that there are some sex-related differences in the concentrations of the examined biogenic amines in the brain in the intact animals, irrespective of the urokinase gene: the females both carrying uPA-/- and uPA+/+ demonstrate in their brains a higher content of adrenaline and histamine, but at the same time a lower level of dopamine and serotonin as compared with the respective data in the males. Similar findings have been obtained by some other researchers, which have recorded, among the other things, elevated levels of serotonin and its metabolite 5-HIAA in the males against those found in the females. This sort of differences in the neuromediator-associated metabolism is responsible for sex-related distinctions in behavioral effects and molecular mechanisms of psychostimulants [24].

CNP produces more changes in the parametric values involved in the experimental studies in the females, when compared with the males, addressing not only the serotonergic system, but also lowering the concentrations of noradrenaline, dopamine ( in the uPA-/- females) and adrenaline (in the uPA+/+ females). The concept on the sex-related specificity in perceiving both acute and chronic pain cannot be treated as a new one: it is well-known that the females demonstrate a higher sensitivity to CNP and produce a more acute responsive effect than it is recorded in the males [25].

The systems responsible for pain suppression with their pathways starting from the spinal cord include the noradrenergic inhibition of the nociceptive transmission in the spinal cord [11]. In our studies, we have shown that the level of noradrenaline decreases as a response to CNP in the brain in the females irrespective of the condition of the urokinase gene and in the uPA-/- males only. The existing opinions on the role of noradrenaline in modulation of chronic pain are rather inconsistent, and a number of investigators believe that after a traumatic damage of the nerve the noradrenergic neurons are no longer inhibitors, but on the contrary, they become chronic neuropathic generators of pain [26]. Although the contribution of the central noradrenergic systems to modulation of the neuropathic pain draws a considerable attention, the adrenoreceptors cannot be treated as "the pure pharmacological target" to suppress the pain syndrome [11].

A deficiency in urokinase in the uPA-/- female/male animals induces an activation of the serotonergic system of the brain as a response to CNP that implies an intensification both of 5 HT metabolism and its synthesis, while in the uPA+/+ males no significant changes in the 5HT and 5-HIAA concentrations have been revealed; in case with the uPA+/+ females we have observed a lowering of the 5HT concentration against the growth of the content of its metabolite 5-HIAA. It is known that transmission of the 5-HT signals can control comorbidity of pain and depression [27]. In this case, serotonin and noradrenaline reuptake inhibitors, which increase extracellular levels of these neurotransmitters, are the key variants of treatment of depression and CNP, and they can favor neurogenesis in the hippocampus [28]. It has been found that the neurometabolic changes, which, on the contrary, result in a decrease in the serotonin level in the hippocampus, produce depressive syndromes according to the model of inflammatory pain in rodents [13].

The absence of a response to CNP by the dopaminergic and histaminergic systems in the brain in the males, irrespective of the urokinase gene, with a decrease in the concentration of dopamine in the uPA-/- females and that of histamine in the uPA+/+ females has engaged our attention. The dopaminergic neurons in the brain ventral region release dopamine and enhance the analgesic activity via D2-like receptors [27]. It should be noted that in the urokinase knockout mice the dopamine level in their brain has significantly exceeded the respective indicators recorded in the uPA+/+ mice, so that even upon a decrease of the concentration of this amine in the brain due to CNP the DA level remains higher than the respective indicators in the uPA+/+ females. At the same time, the level of histamine in the brain declines as a response to CNP in the uPA+/+ females only. It is suggested that there is a close relationship between the histaminergic and dopaminergic systems in the brain, since the histaminergic neurons express dopamine-synthesis ferment DOPA decarboxylase [17]. The presence of the relationship between the histaminergic system and the dopaminergic systems in the brain is supported by researches conducted in patients with Parkinson's disease, when and where an increased histamine concentration in the patients' striatum has been detected. It has been shown that histamine negatively regulates release of dopamine in the striatum [17]. Besides, due to the availability of different subpopulations of histamine neurons, as a result of inhibition of transmission of signals of histamine receptor H3, not only the histamine level in the brain, but also the concentrations of other neurotransmitters may increase, specifically those of serotonin, noradrenaline and dopamine [18]. This sort of research works demonstrates the complexity and multidimensional nature of interactions of the brain neurotransmitters as a response to different actions and stimulation.

As described above, the growth of melanoma B16/F10 B takes place more aggressive in males as compared with females: it is characterized by earlier tumor formation and shortened life duration [29]. As to the dynamics of changes in the neuromediator background in the brain, it should be noted that there are both a sex-related specificity and a dependence

on the urokinase gene condition. In the uPA+/+ and uPA-/- females we have observed an activation of the serotonergic system as a response to the tumor growth that has been reflected in an increase in the concentrations of 5HT and 5-HIAA; however the similar activation in the males have been reported only under the urokinase deficit; in the uPA+/+ males we have detected a suppression of serotoninergic system as a response to the melanoma growth in the brain. We suggest that it is precisely the activation of the serotonergic system in the brain that is one of the factors to increase adaptation capabilities and plasticity of the brain as a response to variable actions and exposures. Dysfunction of the serotonergic system of the suppression type is involved in various pathological states, including depression, ageing, Alzheimer's disease and chronic pain disorders [10]. Our studies demonstrate the participation of this brain system in the pathogenesis of the malignant process as well.

It should be noted that in the uPA+/+ animals the observed changes in the neuromediator status in the brain as a response to the tumor growth have shown two opposing tendencies depending on the sex: in the females we have reported no change in the condition of the noradrenergic, adrenergic and histaminergic systems, but at the same time the dopaminergic system has been activated, while in the males, on the contrary, we have observed the activation of the noradrenergic and adrenergic systems, the suppression of the dopaminergic system with no change in the histaminergic system. In the uPA-/- females and males the tendencies of changes in the dopaminergic, histaminergic and serotonergic systems as a response to the melanoma growth have been found to be identical, and differences have been recorded only in the activation of the adrenergic system in the males, while the uPA-/- females have demonstrated the activation of their noradrenergic system. Probably these are precisely the changes in the activity of the neuromediator systems in the brain as the central systems of the regulation of the anti-tumor resistance in an organism which are one of the factors promoting the suppression of the melanoma growth in the urokinase knockout animals.

It turns out that the most complex process considering its actions and effects is the melanoma growth against the background of CNP. The combination of several stressor factors, when and where each of them has demonstrated both similarity and differences in their actions and effects on the neuromediator status in mice, has resulted in an aggravation of the malignant process in the uPA+/+ animals and cancellation of genetically determined inhibition of the tumor growth in the uPA-/- females/males.

In the uPA-/- females the trend of the recorded changes in the noradrenergic and dopaminergic systems under the combination of the melanoma growth and CNP has corresponded to those changes in the brain of the animals, which have been identified either in case of the CNP action alone or under the uncombined B16/F10 process, while the serotonergic system, on the contrary, has been suppressed, as differentiated from the response to each uncombined acting factor as mentioned above.

In the uPA+/+ females we have found that the tendency of the changes in the biogenic status in the brain under B16/F10+CNP is opposite to those, which are typical for separately acting CNP. In this case, activated have been the adrenergic, noradrenergic and histaminergic systems in the brain, which have not been involved in case with the melanoma growth only.

The combined action of CNP and the melanoma B16/F10 growth has produced its effect on the mediator background in the brain in the uPA+/+ males by inducing the changes in the adrenergic, dopaminergic and serotoninergic systems, similar to those recorded under the melanoma growth alone, with a suppression effect made in parallel on the histaminergic system. In the uPA-/- males we have detected that the melanoma growth against the background of CNP has exerted its effect on all examined brain neuromediator systems other than that found under the action of CNP alone, with alteration of the response by the adrenergic, noradrenergic and serotoninergic systems to the melanoma growth.

## Conclusion

We suggest that one of the factors responsible for cancelation of the genetically determined inhibition of inoculated melanoma B16/F10 combined with CNP progression in the urokinase knockout mice is suppression of the noradrenergic, serotoninergic and dopaminergic systems in the brain, which have been initially activated for compensation of the urokinase deficiency.

## Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

## Conflict of interest

None declared.

## Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

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