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Differences in the functioning of the hypothalamic-pituitary-gonadal axis of regulation in male rats at one (liver) site and two (liver and lungs) sites of metastasis of sarcoma 45 in the experiment

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Abstract

Aims: to study the features of the functioning of the hypothalamic-pituitary-gonadal axis (HPGA) regulation in male rats at the stages of liver metastasizing. **Materials and methods**. Our research work was performed in 30 outbred male rats. Metastases in the liver were produced by implantation of sarcoma 45 (S45) cells into the spleen, which was previously positioned under the skin. The time spans of the study are 5 weeks (the pre-metastatic stage) and 7 weeks (the metastatic stage) after tumor cell transplantation. In the tissues, the content of the following hormones was determined by RIA: luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), total testosterone (Ttot), progesterone (P4); by ELISA we determined the content of the following hormones: gonadotropin-releasing hormone (GnRH), free testosterone (Tfr) and estrone (E1). **Results.** At all stages of the study, the level of GnRH in the hypothalamus decreased by more than 2.0 times; in the pituitary gland, the hormone levels had multidirectional dynamics: LH decreased by 1.6 times, and FSH increased by more than 6.0 times. Liver metastases were characterized by high levels of E1 and Ttot. In the gonads, a high level of P4 was recorded and concentrations of both forms of testosterone were reduced. The concentrations of E2 (by 1.6 times), Tfr (by 4.8 times) increased in blood, and the level of Ttot decreased (by 1.9 times). The salient features of HPGA in the presence of two metastasis sites (liver and lungs) were as follows: in blood, a 2.0 times lower increase in the LH and Tfr contents, a 1.6 times greater increase in E2, an increase in P4 (2.6 times), 1, 4 times lower level of FSH; in the gonads, there are found lower levels of P4, E1, but higher levels of Tfr and Ttot; in liver metastases, a greater increase in P4 (5.2 times), E1 (2.2 times) and Tfr (2.0 times) is recorded. **Conclusion**. Metastasizing to the liver was accompanied by activation of HPGA with the maximum accumulation of reactogenic E1 in liver metastases in rats with two metastasis sites that may indicate their more severe malignancy and ability to metastasize to the lungs.

Keywords

Metastases, Liver, Hypothalamic-pituitary-gonadal axis, Sex steroids, Rats, Males, Sarcoma 45

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Introduction

The hypothalamic-pituitary-gonadal axis, along with the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroid axes, is one of the three main regulatory hormonal systems of the body. It has a central regulatory link, which includes the hypothalamus and the pituitary gland, a peripheral regulatory link, consisting of the gonads, and an effector link, represented by tissues, which are furnished with receptors for sex steroids or peptides and where ectopic synthesis of sex hormones occurs [1, 2]. Gonadotropin-releasing hormone (GnRH) is a decapeptide secreted by the hypothalamic neurons of the arcuate nucleus. It is transported through the axoplasm of nerve endings and flows through the pituitary portal veins to the anterior pituitary gland, where it is supplied by blood to the pituitary cells that secrete luteinizing (LH) and

follicle-stimulating (FSH) hormones. These latter, by regulating the level of sex hormones, make their influence on the formation of gametes and the endocrine system of the gonads [3,4]. In turn, circulating levels of testosterone, estradiol, and some of their metabolites modulate the pulse secretion of GnRH by the hypothalamus directly into the pituitary portal vein system, despite the regulatory effects of GnRH extending to the other organs of HPGA [5].

FSH and LH are tropic pituitary hormones encoded by the same single copying gene [6]. Gonadotropins can exert a carcinogenic effect through a process that has been termed "enhanced hormonal stimulation" of target tissues. It is possible that the procarcinogenic effect made by some peptide hormones is realized with the mediating role of some peripheral hormones controlled by them. Normally, in males, the LH and FSH receptors are mainly limited to gonadal cells [7]. However, under the conditions of malignant pathology, the FSH receptors are found in intratumoral vessels both of the primary tumors (the lungs, the breast, the prostate, the colon, the kidneys, leiomyosarcoma) and metastatic tumors (metastases of the bones, the liver, the lymph nodes, the brain, the lungs, the pleura), and their density differs in different tumors [8].

It is known that sex steroids are the initiators and promoters of carcinogenesis of tumors commonly referred to as hormone-dependent: the tumors of the ovaries, the uterus, the prostate, and the breast. At the same time, it has been established that the pathogenesis of some malignant tumors, which were not previously classified as hormone-sensitive ones, involves the HPGA regulation in colorectal cancer [9], skin melanoma [10] and in some other cases. There are some studies devoted to the research of the functioning of the gonadal axis of the regulation under metastasizing of malignant tumors [11]. However, most works on this subject study the content of individual hormones either in blood or in tumors. Fragmentary information does not allow us to put together a complete picture of the functioning of HPGA under the conditions of oncopathology. It is known that the liver is considered one of the most frequent sites of metastasis of various solid tumors [12]. It is also known that the liver is involved in the metabolism of steroid hormones, which, in turn, make their impact on the metabolic processes in the liver [13]. Based on the above, it seems relevant to study the features of the functioning of HPGA at the stages of metastasizing of malignant tumors to the liver.

The aim of our research work was to study the features of the functioning of the hypothalamic-pituitary-gonadal axis of the regulation in male rats at the stages of metastasizing to the liver.

Material and methods

The experimental study was carried out in white outbred male rats weighing 180-250 grams (n=30), which were delivered by the breeding facility at the National Medical Research Center of Oncology at the Ministry of Health of Russia, and which were kept under the natural light conditions with free access to water and food. Work with animals was conducted in accordance with the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Directive 86/609/EEC), as well as in compliance with the International Guiding Principles for Biomedical Research Involving Animals and Order No. 267 "Approval of the rules of laboratory practice" dated June 19, 2003, issued by the Ministry of Health of Russia.

The rats were divided into groups as listed below: group 1 (reference) where the spleen was positioned under the skin (n=10); group 2 (MTS5) – 5 weeks after the introduction of sarcoma 45 (S45) cells into the spleen (n=11); group 3 (MTS7) – 7 weeks after the introduction of S45 cells into the spleen (n=9). The third group was divided into 2 subgroups as follows: MTS7A covering the rats with metastasis in the liver only (n=6) and MTS7B covering the rats with liver and lung metastasis (n=3). An experimental model of the liver metastases in rats was developed by us earlier [14]. The choice of the timing of the study is due to the fact that week 5 is a stage characterized by the presence of a formed primary tumor in the spleen, preceding the visual appearance of metastases in the liver, while week 7 is a stage characterized by the presence of formed metastases in the liver. Moreover, in some animals, the liver metastases were combined with the lung metastases, which at the 7th week of the development of the malignant process were represented by many miliary nodules located through the thickness layer of the lung parenchyma, while the liver metastases looked like large tumor nodules 18.7–34.6 cm^3 in size, with one-two focal growth.

We used the S45 strain supplied by the Federal State Budgetary Institution "The Russian Cancer Research Center named after N.N. Blokhin" at the Ministry of Health of Russia. Material for transplantation

was obtained from donor rats on days 16-17 of the tumor growth. 3-4 weeks after the positioning of the spleen under the skin, healing of the surgical wound and recovery of the animal, 0.1 ml of a suspension of the S45 tumor cells (at a dilution of $1x10^6$ in physiological saline) was injected intralienally.

The rats were decapitated, trunk blood was collected in dry sterile test tubes without preservatives, and serum was isolated by centrifugation. Homogenates were obtained from the internal organs as given below herein: 2% homogenate (the hypothalamus, the pituitary gland, the prostate) and 10% homogenate (the testes, the liver tissue in rats from the MTS5 group, the liver metastases and the liver tissue surrounding metastases – the parametastatic zone (PZ), in rats from the MTS7A and MTS7B groups). Work with tissues was carried out on ice. In the obtained samples of biological material, the following was studied: by radioimmunoassay (RIA) the content of LH, FSH, estradiol (E2), total testosterone (Ttot), progesterone (P4) (Immunotech, Czech Republic); by the ELISA method the concentrations of GnRH, free testosterone (Tfr), estrone (E1) and sex steroid binding globulin (SSBG) (Casabio, China). Statistical processing of the obtained results was completed using the parametric Student's test with a personal computer employing the STATISTICA 12.0 software and the non-parametric Wilcoxon-Mann-Whitney test, having previously checked the data sets on normality of distribution of the indicators (Shapiro-Wilk test for small samples). Table data are presented herein as M±m, where M is the arithmetic mean, m is the standard error of the mean. Differences between two samples were considered statistically significant at $p<0.05$.

Results

It was found that in the rats with the model of metastatic liver damage, compared with the reference, the content of GnRH in the hypothalamus decreased in the MTS5 group by 2.0 times, in the MTS7A group by 2.2 times, and in the MTS7B group by 2.6 times (see Table 1 herein).

The dynamics of concentrations of tropic hormones in the pituitary gland was multidirectional: the level of LH at the stages of metastasis decreased by 1.6-1.7 times compared with the reference, while the content of FSH, on the contrary, increased in the MTS5 group by 6.3 times, in the MTS7A group by 6.5 times, and in the MTS7B group by 6.8 times, respectively (see Table 1 herein). It should be noted that, unlike the pituitary gland, the concentrations of LH and FSH in blood depended on the stage of the study and the presence of metastases in the lungs. Thus, the content of LH in blood of the rats from the MTS5 group did not differ from the reference, while at the stage of metastasis it increased in the MTS7A group by 4.6 times, and in the MTS7B group by 2.1 times compared with the reference and the pre-metastatic stage; the difference between the groups of the rats with formed liver metastases, but with different status of lung metastases, was 2.2 times (see Table 1 herein). The level of FSH in blood, on the contrary, decreased in the MTS5 and

Table 1

The content of GnRH in the hypothalamus, FSH and LH in the pituitary gland and blood serum in male rats at the stages of metastasizing

Indicator	Reference	MTS5	MTS7	
			A	B
GnRH in hypothalamus (ng/g tissue)	0,041 ±0,004	0,020^{+} ±0,004	0,019^{+} ±0,003	0,016^{+} ±0,003
LH in pituitary gland (IU/g tissue)	0,019 ±0,000	0,012^{+} ±0,002	0,012^{+} ±0,002	0,011^{+} ±0,001
LH in blood serum (IU/L)	0,18 ±0,05	0,18 ±0,02	0,83$^{+,5}$ ±0,05	0,38$^{+,5,7}$ ±0,02
FSH in pituitary gland (IU/g tissue)	0,0010 ±0,000	0,0063^{+} ±0,000	0,0065^{+} ±0,0002	0,0068^{+} ±0,0003
FSH in blood serum (IU/L)	8,83 ±0,65	2,74^{+} ±0,46	8,32^{5} ±0,65	5,98$^{+,5,7}$ ±0,44

Notes: Statistically significant differences from the following data: $^{+}$ from the reference; ^{5} from the MTS5 data; ^{7} from the MTS7A data.

MTS7B groups by 3.2 times and 1.5 times, respectively, while in the MTS7A group it did not statistically significantly differ from the reference; the difference between the MTS7A and MTS7B groups was 1.4 times (see Table 1 given herein).

The content of P4 in the liver of the rats in the MTS5 group decreased compared with reference values by 3.2 times (see Table 2 herein). In the rats in the MTS7A group, the level of the hormone in the metastasis and PZ corresponded to the reference values. In the rats in the MTS7B group, the content of P4 increased sharply and became greater than in the reference group: in the metastasis by 3.6 times and in PZ by 11.3 times, correspondingly, and compared with the values of the MTS5 group it increased in the metastasis by 11.4 times and in PZ by 35.8 times, respectively; as compared with the corresponding tissues of the rats in the MTS7A group in the metastasis by 5.2 times and in PZ by 17.4 times, correspondingly (see Table 2 herein).

The content of E1 in the liver of the rats in the MTS5 group did not statistically significantly differ from the reference values, while in the rats at the stage of the formed liver metastases it increased, reaching greater values in the metastases than it was the case with the surrounding tissues, especially in the rats in the MTS7B group (see Table 2 herein). Thus, the concentration of E1 in the liver metastasis was higher than that measured in the liver tissue in the reference rats and the rats from the MTS5 group: in the MTS7A group by 6.0 times and 8.3 times, respectively, and in the MTS7B group by 13.2 times and 18.1 times, respectively; the difference in the content of the above hormone in the liver metastases in the rats with different types of metastasis was 2.2 times (see Table 2 herein). The level of E1 in PZ was the same in all rats: less than in the corresponding metastases, namely, in the rats in the MTS7A group by 3.7 times and in the rats in the MTS7B group by 8.1 times, but it was 1.6 times higher than in the reference group and 2.2 times higher than it was the case with the MTS5 group.

The level of E2 increased in comparison with the reference values by 1.1 times in the liver tissue only in the rats in the MTS5 and MTS7A groups (see Table 2 herein). The content of Tfr became higher than the reference values in the liver in the rats in the MTS5 group by 3.4 times, as well as in metastasis and PZ by 1.8 times in the rats in the MTS7B group. The difference in the level of Tfr between the MTS7A and MTS7B rats in their metastases was 1.8 times and that recorded in PZ was 2.0 times (see Table 2 given herein). The content of Ttot in the liver tissues increased at all stages of metastasizing: in the rats in the MTS5 group by 15.0 times, in the rats in the MTS7A and MTS7B groups by more than 200.0 times as against the corresponding reference indicator. As a result, the level of Ttot in the malignant and surrounding liver tissue was more than 13.0 times higher than at the previous stage of the study (see Table 2 given herein).

In the reproductive organs at the stage of the formed metastases, the content of P4 increased as compared with the reference as follows: in the MTS7A group only in the testes by 20.1 times; in the MTS7B group and in the testes by 10.0 times, and in the prostate by 6.5 times (see Table 3 herein). It should be noted that in the prostate in 60% of the rats from the MTS5 group, the level of P4 decreased by 22.0 times, and in 40% of the cases it increased by 2.4 times com-

Table 2

The content of sex steroids in the liver (per gram of tissue) at the stages of metastasizing

	Reference	MTS5	MTS7A		MTS7B	
			M	PZ	M	PZ
P_4 (ng)	2,31±0,30	0,73^{+} ±0,15	1,60^{5}±0,20	1,50^{5}±0,28	8,30$^{+,5}$±0,95	26,10$^{+,5,7A,m}$±0,31
E_1 (pg)	75,45±9,59	54,74±4,84	454,5$^{+,5}$±53,21	122,13$^{+,5,m}$±8,23	993,42$^{+,5,7A}$±70,25	122,84$^{+,5,7Am,m}$±13,45
E_2 (ng)	1,70±0,10	1,92^{+}±0,03	1,62^{5}±0,05	1,91$^{+,м}$±0,05	1,62^{5}±0,04	1,52^{5,7Ap}±0,05
Tfr (pg)	1,31±0,10	4,45^{+}±1,01	1,34^{5}±0,21	1,18^{5}±0,18	2,42^{7Am}±0,28	2,34^{7Ap}±0,54
Ttot (ng)	0,01±0,00	0,15^{+}±0,03	2,00$^{+,5}$±0,10	2,20$^{+,5}$ ±0,16	2,30$^{+,5}$±0,42	2,00$^{+,5}$±0,31

Notes: Statistically significant differences from the following data: $^{+}$ from the reference data, ^{5} from the MTS5 data, ^{7A} from the data in metastases and parametastatic zone of rats in the MTS7A group, ^{7Am} from the data in metastases of rats in the MTS7A group, ^{7Ap} from the data in parametastatic zone in the rats from the MTS7A group, ^{m} metastasis within the same group. M – metastasis, PZ – parametastatic zone.

pared with the reference group, while in the testes at that stage of the study, the content of P4 demonstrated no changes (see Table 3 given herein). In the rats in the MTS7B group, the level of E1 also decreased in the testes by 6.5 times, and in the prostate it fell to 0, while in the rats in the MTS7A group the content of E1 did not change in the testes or was increased by 4.3 times compared with the reference in the prostate (see Table 3 herein). The content of E2 decreased in the testes by 1.3 times and in the prostate by 1.2 times already at the pre-metastatic stage, and at the stage of the formed metastases in the testes in all rats it was restored to the reference level. In the prostate, the recovery of the E2 content was observed only in the rats in the MTS7B group, while in the rats in the MTS7A group the hormone level remained low (see Table 3 given herein).

Only at the stage of the formed metastases the content of both forms of testosterone and SSBG did change in the gonads of rats. Thus, the concentration of Tfr decreased both in the testes and in the prostate in all rats: in the MTS7A group by 14.4 times and 7.4 times, respectively, and in the MTS7B group by 3.6 times and 62.6 times, respectively. Consequently, in the rats in the MTS7A group, the testes contained 4.0 times less, and in the prostate 8.5 times more Tfr than in the corresponding organs of the rats in the MTS7B group (see Table 3 herein). The level of Ttot, as well as Tfr, decreased in the testes of the rats in the MTS7A group by 6.3 times and in the MTS7B group by 2.2 times and increased in the prostate by 16.2 times and 15.4 times, respectively, as against the previous study period. The difference in the above indicator in the testes between the MTS7A and MTS7B groups was 2.9 times (see Table 3 herein). The content of SSBG in the rats in the MTS7A group increased in the gonads by 2.3 times, and in the rats from the MTS7B group, on the contrary, it decreased in the testes by 1.9 times, in the prostate by 3.8 times compared with the reference. The difference between the groups in the SSBG content in the testes was 4.4 times and that in the prostate was recorded to be 8.9 times (see Table 3 herein).

In blood of the rats in the MTS5 group, the concentrations of only E2 and Tfr changed. In the first case, the content of the hormone decreased by 1.4 times, and in the second case it increased by 46.3 times compared with the initial level (see Table 4 given herein). In the rats with the formed metastases, regardless of the presence or absence of a second metastasis site, the serum content of E2 increased, while the content of Tfr and Ttot decreased compared with the previous stage of the study. The level of E2 became higher than that at the previous stage of the study and in the reference group: in the rats in the MTS7A group 2.3 times and 1.6 times higher, respectively, and in the rats in the MTS7B group 3.6 times and 2.5 times higher, accordingly; the difference in the above indicator between

Table 3

The content of sex steroids and SSBG in the testes and prostate (per gram of tissue) at the stages of liver metastasis

	Testes					Prostate			
	Reference	MTS5	MTS 7A	MTS 7B		Reference	MTS5	MTS 7A	MTS 7B
P_4 (ng)	0,88 ±0,26	1,44 ±0,24	17,7$^{+,5}$ ±2,50	8,8$^{+,5,7A}$ ±0,99	P_4 (ng)	1,76 ±0,70	1,87±0,87 ↙ ↘ 0,08 ±0,05 4,27 ±0,48	3,60^{5} ±0,05	11,50$^{+,5,7A}$ ±0,12
E_1 (ng)	0,71 ±0,12	1,15 ±0,22	0,90 ±0,15	0,11$^{+,5,7A}$ ±0,05	E_1 (ng)	0,33 ±0,09	1,65^{+} ±0,15	1,41^{+} ±0,09	0$^{+,5,7A}$
E_2 (ng)	1,99 ±0,10	1,49^{+} ±0,14	1,96^{5} ±0,22	2,03^{5} ±0,18	E_2 (ng)	1,84 ±0,08	1,56^{+} ±0,08	1,47^{+} ±0,08	1,86^{7A} ±0,05
T*fr* (ng)	0,72 ±0,02	0,67 ±0,07	0,05$^{+,5}$ ±0,00	0,20$^{+,5,7A}$ ±0,01	T*fr* (ng)	31,95 ±9,55	17,16 ±4,90	4,31$^{+,5}$ ±0,24	0,51$^{+,5,7A}$ ±0,24
T*tot* (ng)	17,64 ±3,22	10,77 ±1,13	1,70$^{+,5}$ ±0,20	5,00$^{+,5,7A}$ ±0,67	T*tot* (ng)	0,26 ±0,07	0,13 ± 0,04	2,10$^{+,5}$ ±0,18	2,00$^{+,5}$ ±0,25
SSBG (nMol)	5,36 ±0,80	5,00 ±0,26	12,40$^{+,5}$ ±1,16	2,80$^{+,5,7A}$ ±0,52	SSBG (nMo)	5,36 ±0,80	5,75 ±0,46	12,43$^{+,5}$ ±2,22	1,40$^{+,5,7A}$ ±0,35

Notes: Statistically significant differences from the following data: $^{+}$ from the reference data, ^{5} from the MTS5 data, ^{7A} from the MTS7A data.

the MTS7A and MTS7B groups was recorded to be 1.6 times (see Table 4 given herein). The content of Tfr and Ttot decreased as against the group of animals MTS5: in the group of the MTS7A rats by 9.7 times and 2.7 times, respectively, and in the group of rats MTS7B by 18.5 times and 3.2 times, respectively, while the level of Tfr remained 4.8 times higher than the initial values in the MTA7A group and 2.5 times higher in the MTS7B group, and the content of Ttot decreased twofold compared with the corresponding reference indicator in all rats with the formed liver metastases (see Table 4 herein). The serum level of P4 decreased in the rats in the MTS7A group by 2.3 times, compared with the 5th week of metastasis, up to reaching the reference values, while in the rats in the MTS7B group it increased both compared with the initial level by 2.6 times and compared with the values in the rats in the MTS5 and MTS7A groups, by 1.7 times and 3.8 times, respectively (see Table 4 given herein). The dynamics of the SSBG concentrations in blood of the rats with formed liver metastases depended on the presence or absence of metastases in the lungs: in the absence thereof, the protein content increased by 1.5 times, while in the presence thereof it decreased by 1.3 times compared with the reference (see Table 4 given herein).

Discussion

How metastasizing of malignant tumors occurs remains a long-standing unsolved problem. There is still no complete understanding of the mechanism of this phenomenon. One of the main questions today remains the dilemma: whether metastases can metastasize secondarily or not. There are two main hypotheses offered to explain the process of metastasizing. One of them is that metastases occur simultaneously at the beginning of the development of the primary tumor and represent the end point of the tumor progression: it is a parallel model of metastasizing [15]. Another is that the primary tumor goes through several stages of its genetic changes before malignant cells acquire the ability to metastasize, so the metastases, which have appeared as the most malignant, in turn, acquire the ability to give metastatic seeding to other organs: it is a linear model [16]. Analyzing our data, we tend to believe that when modeling experimental metastasis of S45 in male rats, the linear model of metastasizing is applicable. This is indicated not only by the much smaller, compared with liver metastases, sizes of metastatic foci in the lungs, but also by the features of the functioning of HPGA regulation, which will be discussed below.

So, the process of S45 metastasis to the liver, apparently, was accompanied by activation of the central HPGA link with an increase in the production of LH and FSH in the pituitary gland. That was evidenced by a decrease in the levels of GnRH in the hypothalamus and LH in the pituitary gland, as well as an increase in the pituitary concentration of FSH at all stages of the experimental study. The revealed changes in the central regulatory link of HPGA were combined with an increase in the serum concentration of LH and an increase in the low level of FSH in blood, more so in rats with metastases only in the liver. The reason for the different directions of the dynamics of tropic hormones in the pituitary gland and different degrees of their increase in the blood could be the following: a different degree of increase in their pituitary synthesis, a disorder in the release

Table 4

The content of sex steroids and SSBG in blood serum (per milliliter) at the stages of liver metastasis

	Reference	MTS5	MTS7A	MTS7B
P_4 (ng)	11,08±1,47	17,35±2,37	$7,50^{5}$±0,09	$28,67^{+,5,7A}$±3,11
E_1 (ng)	9,22±0,29	12,79±1,34	12,52±1,05	$14,66^{+}$±1,69
E_2 ngr)	61,78±2,62	$43,60^{+}$±4,95	$100,0^{+,5}$±6,50	$155^{+,5,7A}$±7,89
Тсв (нг)	0,04±0,01	$1,85^{+}$±0,53	$0,19^{+,5}$±0,02	$0,10^{+,5,7A}$±0,01
Ttot (ng)	0,79±0,11	1,12±0,17	$0,42^{+,5}$±0,05	$0,35^{+,5}$±0,04
SSBG (nMol)	0,62±0,03	–	$0,95^{+}$±0,05	$0,47^{+,7A}$±0,03

Notes: Statistically significant differences from the following: $^{+}$from the reference values, ^{5} from the MTS5 values, ^{7A} from the MTS7A values.

of FSH from the pituitary gland into the bloodstream, different binding affinity to effector receptors in peripheral tissues, including the gonads. It has been established that an increase in the production of FSH in the pituitary gland up to a hundred times greater than LH can be facilitated by the intracerebroventricular expression of the KiSS-1 peptide, which has been originally identified as a metastasis suppressor gene [17]. Whatever it might be, during the period of the formed metastases, there was an increase in the concentration of tropic hormones of the pituitary gland in blood, which regulate the processes of synthesis of sex steroids in the body.

In the liver, before the identifiable appearance of metastases therein, an increased production of androgens was recorded, and the synthesis of E2 was activated that, together with the increase in cortisol, found previously [18], created the conditions for the formation of a metastatic field, within which metastases subsequently developed. Moreover, E2 and cortisol at that stage were synthesized directly in the liver tissue *de novo* from cholesterol. It is known that steroids synthesized locally in tissues, without getting into the bloodstream, can have a local immunosuppressive effect, thereby promoting the tumor cell invasion and metastasis [19].

At the stage of the formed metastases, E1 is accumulated in the liver: more in the metastases than in the parametastatic zones, that is especially the case with the rats with a double metastasis site. The accumulation of E1 in the liver metastases in the rats without lung metastases might be due to several mechanisms, one of which is E1 synthesis in metastases and *de novo* parametastatic zones (an increase in the content of E1 and P4). The replenishment of the E1 level in the metastases probably occurs not so much due to its synthesis in the metastases themselves, but rather due to the synthesis thereof in the parametastatic zones of the liver and subsequent entry into the metastasis (lower E1 level and higher E2 level recorded in the parametastatic zones). Another possible mechanism for increasing the amount of E1 in the liver metastases is the process of aromatization of androgens delivered there from the reproductive organs. This mechanism confirms, on the one hand, a low level of Tfr and a high level of Tot both in the metastases and in the parametastatic zones, and, on the other hand, a feature of steroidogenesis in the testes and prostate. So, in the testes, against the background of increasing levels of P4 and E2, indicating an enhancement of the synthetic processes in the organ, a sharp decrease in the content of all testosterone fractions was recorded, which, together with a decrease in the content of Tfr and an increase in Ttot in the prostate, can be regarded as a discharge of testosterone, newly synthesized by the testes, via the seminal duct into the prostate gland, binding it there with carrier proteins (albumin) and release into the general circulation.

In the rats with two sites of metastasis, the reason for the greater accumulation of E1 in the liver metastases could be more active local synthesis of E1 *de novo* (higher levels of E1 and its precursor P4) and the conversion of greater amount of Tfr delivered from the reproductive system (greater level of Tfr than in the animals with isolated metastasis, against the background of the same high level of Ttot in metastases and the parametastatic areas of the liver), due to a more pronounced testosterone-synthesizing function of the testes (a smaller degree of increase in P4, a significant decrease in E1, a greater, but however remaining low, content of Tfr and Ttot). In the parametastatic zones, the content of E1, which is lower than that in the metastases, in combination with a higher level of P4, might be due to the lower activity of enzymes involved in the synthesis of E1 *de novo*.

Greater testosterone genesis in the testes of the rats with metastases both in the liver and the lungs was combined with a decrease in the content of SSBG in the reproductive organs and blood, while in the rats with metastases only in the liver, their lower testosterone-synthesizing activity was combined with an increase in the content of SSBG in their reproductive organs and blood serum against the background of an almost equivalent increase in the concentration of Ttot in the prostate in all rats. SSBG is a complex protein with multiple functional forms, while its affinity to hormones is partially regulated through proteolysis or binding of non-steroidal ligands [5; 20]. Testosterone, synthesized in the testes, normally binds both to albumin and SSBG. An increase in the concentration of SSBG in the rats without lung metastases indicated the predominant binding of testosterone to albumin, while a decrease in the SSBG concentration in the rats with the presence of lung metastases demonstrated the predominant binding of testosterone to SSBG. It has been established that albumin binds steroids with limited specificity and low affinity, therefore, it easily loses its connection with them, thereby replenishing

their concentration in blood. SSBG, on the contrary, binds steroids with high affinity and specificity, and it is more difficult to part with them, and it purposefully delivers them to target organs [21]. Apparently, it was just the mechanism that determined the higher serum concentration of Tfr in the rats from the group with metastases only in the liver and the higher concentrations of Tfr in the liver metastases and their parametastatic zones in the rats with a double metastasis site. SSBG not only controls the balance of sex hormones, but can also be considered as an enhancer of the effects of sex steroids in general [22]. The lower concentration of Tfr in blood of the rats with two metastasis sites indirectly indicated a greater degree of damage to the vascular endothelium [23], which could be one of the factors contributing to distant metastasizing to the lungs.

Thus, one of the mechanisms contributing to the secondary metastasizing of the liver metastases to the lungs is a greater accumulation of reactogenic procarcinogenic E1 in the liver metastases, resulting from the activation of its probable local synthesis *de novo*, greater stimulation of testosterone genesis in the gonads, and the specifics of the transport of sex steroids by binding to the SSBG and the features of functioning of the central link of the regulation of HPGA.

Conclusions

The process of metastatic damage to the liver by S45 cells is accompanied by the activation of HPGA, beginning with the central link of the regulation, an enhancement of the testosterone-synthesizing function of the testes, and an increase in the processes of testosterone aromatization with a predominant accumulation of E1 in the liver metastases. A higher level of reactogenic E1 in the liver metastases in the rats with two metastasis sites against the background of greater HPGA activity suggests a more malignant nature of the liver metastases, which with a high degree of probability could give secondary metastatic disseminations to the lungs.

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