Disorders in connections of the hypothalamic-pituitary-gonadal axis in rats with Guerin’s carcinoma against the background of induced hypothyroidism


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Abstract

Aims. To study the content of regulatory peptides in the hypothalamus and pituitary gland, as well as sex steroids in the gonads in rats of both sexes with Guerin’s carcinoma against the background of hypothyroidism.

Materials and methods. Our experiment was performed in white outbred rats of both sexes with induced hypothyroidism, against the background of which Guerin’s carcinoma was transplanted. In 1% homogenates of the pituitary and the hypothalamus and in 10% homogenates of the gonads in the animals from the main and reference groups, the levels of GnRH (Casabio, China) were determined by the ELISA method using standard kits, and the content of LH, FSH, estradiol (E2), testosterone (T) and progesterone (P4) by the RIA method (Immunotech, Czech Republic). Statistical analysis of the results was performed using the Statistica 10.0 software package.

Results. In females of the main group, the level of GnRH in the hypothalamus increased by 1.6 times, but the content of LH and FSH in the pituitary gland decreased, while in males the content of GnRH decreased by 1.4 times and FSH by 1.6 times compared with the intact animals. In the gonads of females of the main group, the content of E2 and T decreased by 1.3 times, but the level of P4 increased by 4.7 times, while in males the T production decreased by 7.1 times.

Hypothyroidism, against the background of which Guerin’s carcinoma developed, had a significant effect both on the central and peripheral axes of the hypothalamic-pituitary-thyroid (HPT) and hypothalamic-pituitary-gonadal (HPG) axes, which can lead to additional hormonal imbalance in sex steroids.

Keywords

Hypothyroidism, Guerin’s carcinoma, Rats, Hypothalamic-pituitary-gonadal axis

Introduction

It is known that both thyroid and sex hormones can directly bind to nuclear receptors and act as transcription factors, mediating intracellular effects [1,2,3]. At the same time, the thyroid hormone receptors are able to induce crosstalk with steroid hormone receptors through incomplete elements of the hormonal response or coactivators, including androgen receptors, glucocorticoid receptors, mineralocorticoid receptors, and progesterone receptors [4,5]. In addition, thyroid hormones, their transporters and nuclear receptors have been found in the gonads [6].

The hypothalamic-pituitary-thyroid (HPT) and hypothalamic-pituitary-gonadal (HPG) axes are physiologically connected and act together as a single system under various physiological and pathological conditions [7]. This is due to the general mechanisms of central regulation, when the thyrotropin-releasing hormone of the hypothalamus can make an influence not only on the synthesis of pituitary TSH, but also gonadotrophic hormones LH and FSH [8,9]. Due to the cross interaction of the main regulatory axes of the body, the thyroid hormones control reproductive dysfunctions, so abnormal sexual development, infertility, or cycle disorders may be associated with thyroid pathology [10].

In addition, it is known that pituitary TSH is a heterodimer and consists of alpha and beta polypeptides. The
alpha subunit is common with other pituitary hormones, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and human choriionic gonadotropin (HCG), while the beta subunit is specific for TSH and determines an interaction with the TSH receptor [11]. An imbalance between the main regulatory axes leads to pathological reactions by the body [12].

In our experimental studies, it was shown that induced hypothyroidism in female rats caused a decrease in the absolute volume of the endometrium and a reduction in its muscle layer, and in women, hypothyroidism could result in menstrual irregularities and ovulation [1, 13]. In men, thyroid hormones also affect the functioning of the gonadal axis, in particular T3, regulate the proliferation and differentiation of Sertoli cells, induce differentiation of Leydig cells, and stimulate steroidogenesis that has been demonstrated in some experimental and clinical studies [14, 15].

Since the HPT axis is associated with the reproductive system, and since we previously established the sex specificity of the effect of hypothyroidism on the growth of Guerin's carcinoma [16], it is of particular interest to study changes in the functioning of the HPG axis in animals of both sexes with Guerin's carcinoma growing against the background of a simulated thyroid hormone deficiency.

**The aim** hereof was to study the content of regulatory peptides in the hypothalamus and the pituitary gland, as well as sex steroids in the gonads in rats of both sexes with Guerin's carcinoma against the background of hypothyroidism.

**Materials and methods**

The experiment was performed in white outbred rats of both sexes weighing 150–180 g. The animals were delivered by the Federal State Budgetary Institution Scientific Center for Biomedical Technologies at the Federal Medical and Biological Agency (Andreevka Branch, Moscow Region). The laboratory animals were kept under natural light conditions with free access to water and food. Work with animals was carried out 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 accordance 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.

Animals were manipulated in a box in compliance with the generally accepted rules of asepsis and antisepsis. Reference: Record No. 25/129 dated 10/27/2021 prepared by our Ethics Committee.

We used a strain of Guerin's carcinoma, 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 12-16 of the tumor growth. Transplantation of Guerin's carcinoma to animals was carried out by standard subcutaneous injection of a tumor suspension under the skin of the right shoulder blade in a volume of 0.5 ml of cell suspension diluted in saline 1:10.

The white outbred rats of both sexes received for 30 days pharmacopeial thyreostatic drug Merkazolil ("Akrikhin" Russia) at a daily dose of 2.5 mg/100 g of body weight (the total dose was 75 mg/100 g of body weight). The animals did not refuse to eat, gained weight, and no deterioration in the appearance of the skin and the hairline, no lethargy or drowsiness were recorded. Hypothyroidism in animals was confirmed by determining the content of total T4 and TSH in blood serum, by radioimmunoassay using standard kits (Immunotech, Czech Republic) 30 days after taking the thyreostatic agent. The animals of each sex were divided into groups of 15 females and 15 males: The main group was composed by the rodents after persistent hypothyroidism, and the animals therein were subcutaneously transplanted with Guerin's carcinoma. Reference group No. 1 covered the animals with hypothyroidism. Reference group No. 2 included the animals without hypothyroidism, with an independent growth of Guerin's carcinoma only. The intact animals (10 females and 10 males) were used as those showing the physiological norm.

The animals were decapitated (reference group No. 2 and the main group after 18 days of growth of Guerin's carcinoma). In 1% homogenates of the pituitary and the hypothalamus and in 10% homogenates of the gonads, the level of GnRH (Casabio, China) was determined by the ELISA method using standard kits and the content of LH, FSH, estradiol (E2), testosterone (T) and progesterone (P4) by the RIA method (Immunotech, Czech Republic).

Our statistical analysis of the results was performed using the Statistica 10.0 software. The data obtained were analyzed for the compliance of the distribution of signs with the normal distribution law using the
Shapiro-Wilk test (for small samples). Comparison of quantitative data in the groups was performed using Student’s t-test and Mann-Whitney test. Table data are presented herein as M±m, where M is the arithmetic mean, m is the standard error of the mean; p<0.05 was taken as the level of statistical significance.

**Results**

Previously, we found that induced hypothyroidism inhibited the growth of transplanted Guerin’s carcinoma and increased life expectancy only in female rats, while in male rats, despite the fact that the size of the tumors was smaller at the initial stages, after 2 weeks of the experiment it was comparable with the parameters in the animals with the independent tumor growth, and no increase in life expectancy was observed [16,17]. An analysis of the content of hypothalamic Gn-releasing and pituitary tropic hormones LH, FSH and prolactin (PRL) in rats of both sexes is presented in Table 1 herein.

It was found that in the intact animals there were some gender differences in the content of the studied parameters: the level of GnRH in the hypothalamus was 2 times higher in the males, and the levels of LH and FSH in the pituitary gland in the females were 1.4 times higher (p<0.05) and 2.9 times, respectively. The level of prolactin in the pituitary gland of intact animals had no gender differences.

It was found that in the female rats of the reference group No. 1 (with hypothyroidism), the content of GnRH in the hypothalamus and FSH in the pituitary gland was 1.8 times and 3.9 times lower, respectively, and PRL was 1.3 times higher, and only the concentration of LH had no significant differences compared with the intact animals.

The growth of Guerin’s carcinoma in the independent variant (reference No. 2), compared with the intact animals, affected the levels of GnRH in the hypothalamus and FSH in the pituitary gland, increasing the former by 1.4 times and reducing the latter by 2.1 times, respectively, but that did not change concentration of LH and PRL in the pituitary gland. As a result, the level of in the hypothalamus of the females with the independent tumor growth was 2.4 times higher, and the level of FSH was 1.9 times higher, while prolactin, on the contrary, was 1.6 times lower compared with the animals with hypothyroidism.

In the females of the main group, against the background of a 1.6-fold increase in the level of GnRH as compared with intact animals, a decrease in the content of LH by 1.25 times and FSH by 7.5 times was detected, but at the same time an increase in PRL by 1.3 times was found. Therewith, in the main group, the females had the lowest level of tropic hormones in the pituitary gland and the highest GnRH release compared with the reference groups.

In the males with hypothyroidism (reference group No. 1), a decrease in the level of GnRH in the hypothalamus by 1.4 times was found, compared with the indices in the intact animals, while multidirection-

<table>
<thead>
<tr>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypothalamus</strong></td>
<td><strong>Pituitary gland</strong></td>
</tr>
<tr>
<td>GnRH release ng/g tissue</td>
<td>LH mIU/g tissue</td>
</tr>
<tr>
<td>Intact animals (norm)</td>
<td>0.28±0.03</td>
</tr>
<tr>
<td>Reference No.1</td>
<td>0.16±0.02</td>
</tr>
<tr>
<td>Reference No.2</td>
<td>0.39±0.04</td>
</tr>
<tr>
<td>Main group</td>
<td>0.46±0.05</td>
</tr>
</tbody>
</table>

Notes: significant differences compared with the following: ‘with the indicators of intact animals; ‘compared with reference group No.1; ‘compared with reference group No.2.

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al changes in tropic hormones in the pituitary gland were recorded: LH decreased by 1.25 times and PRL by 2 times, and FSH did not change, compared with the indicators in the intact animals.

In the males with Guerin’s carcinoma (reference group No. 2), compared with the intact animals, an increase in the content of GnRH in the hypothalamus by 2.8 times and FSH in the pituitary gland by 1.3 times was revealed, while a decrease in the level of LH and PRL in the pituitary gland on average 1.3 times was reported. Compared with the parameters in animals of the reference group No. 1, the growth of Guerin’s carcinoma had a different effect on the level of GnR-releasing hormone, and its concentration in the hypothalamus was 3.9 times higher than in hypothyroidism; the content of LH and FSH in the pituitary gland in reference groups No.1 and No.2 had no significant differences, while the level of prolactin was 1.5 times higher in the animals with Guerin’s carcinoma.

In the main group, in the males, compared with the intact animals, against the background of a 1.4-fold decrease in GnRH in the hypothalamus, a 1.6-fold decrease in the level of FSH and a 1.3-fold decrease in PRL in the pituitary gland were recorded, without changing the content of LH. As a result, the males of the main group had the lowest level of FSH in the pituitary compared with the reference groups.

Those results showed that in the females of the main group, the hypothalamic corresponded according to the direction of changes to the relevant indicators in the animals with the independent tumor growth, while in the males of the main group, on the contrary, it corresponded to the indicators in animals with independent hypothyroidism.

Next, we studied the content of sex hormones in the gonads in animals of both sexes with the independent Guerin’s carcinoma growth and that combined with hypothyroidism (see Table 2 herein).

It was found that in the females with hypothyroidism, compared with the intact animals, the level of estradiol in the ovaries increased by 2.1 times and that of progesterone by 2.6 times, against the background of a decrease in the testosterone levels by 1.7 times. In the females of the reference group No.2, on the contrary, the level of E2 in the ovaries was 2.6 times lower than the normal one, and the level of testosterone was 2.3 times lower, against the background of the elevated progesterone level reported to be 2.7 times higher. In

Table 2
The content of steroid hormones in the gonads in rats (pg/g tissue)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ovaries, females</th>
<th>Testes, males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Е2</td>
<td>T</td>
</tr>
<tr>
<td>------------------</td>
<td>----</td>
<td>---</td>
</tr>
<tr>
<td>Intact animals</td>
<td>134,6±11,4</td>
<td>3,5±0,36</td>
</tr>
<tr>
<td>Reference No.1</td>
<td>279,1±28,0</td>
<td>2,1±0,28</td>
</tr>
<tr>
<td>Reference No.2</td>
<td>50,95±4,6</td>
<td>1,5±0,2</td>
</tr>
<tr>
<td>Main group</td>
<td>102,4±6,2</td>
<td>2,7±0,24</td>
</tr>
</tbody>
</table>

Notes: significant differences compared with the following: 1 with the indicators of intact animals; 2 compared with reference No.1; 3 compared with reference No.2 (р˂0.05).
the main group, the content of E2 was 1.3 times lower compared with the intact animals, and P4 exceeded their values by 4.7 times. At the same time, in the main group, the level of progesterone exceeded the indicators recorded in the reference groups.

In the males with hypothyroidism, the concentration of estradiol and testosterone in the testes turned out to be below the norm by an average of 1.6 times, and that of progesterone, on the contrary, was 1.5 times higher. The growth of Guerin's carcinoma caused a decrease in the content of all studied steroids: E2 by 1.3 times, T by 1.8 times and P4 by 1.4 times, respectively. In the main group, the level of E2 in the testes did not have significant differences from the norm, T was reduced by 7.1 times, and P4, on the contrary, increased by 1.3 times. As a result, in the males in the main group, the testosterone content was the lowest among all the studied groups.

Discussion

There are many hypotheses on the effect of thyroid dysfunction on reproduction, and the junction may be the highest level of the regulatory axes that affects the activity of the brain neurons, the hypothalamus, and the pituitary gland [18].

Our study showed that the change in the level of GnRH in the hypothalamus in rats of the reference groups had no gender specificity: it decreased in the animals of both sexes against the background of hypothyroidism, while it increased during the growth of Guerin's carcinoma. However, it is known that the combination of two pathologies can affect different pathways of regulation that is why the presence of comorbid pathology complicates and modifies the course of the primary disease [19]. In the main group, the gender differences were revealed in the direction of changes in the level of GnRH of the hypothalamus: in the females, induced hypothyroidism inhibited the increase in volumes, and the level of GnRH in the hypothalamus increased, similar to the processes in reference group No. 2, while in the males of the main group, on the contrary, it decreased, while the recorded tumor volumes and life expectancy did not differ from those in reference group No. 2.

It should be noted that there is a small number of studies dealing with the issue of the central regulation of the axes. However, there are some experimental studies, the results of which indicate the involvement of both central regulatory factors, such as LH and FSH, and peripheral sex steroids in the development of a malignant tumor [20].

In addition, attention should be drawn to the fact that in the females, both in the reference and in the main groups, a decrease in the level of FSH in the pituitary gland was detected, as a result of which LH turned out to be the predominant tropic hormone, while in males, a decrease in the level of FSH in the pituitary gland was recorded in the case of an increase in Guerin's carcinoma against the background of hypothyroidism only. That is, in our study, it has been revealed that the production of releasing hormones by the hypothalamus and tropic hormones by the pituitary gland changes in response to tumor growth and hypothyroidism, depending on the sex of the animal.

There are reference literature data indicating that an imbalance in the ratio of tropic hormones and the prevalence of one over another can lead to some pathological reactions, in particular as follows: an increase in the FSH levels can be one of the unfavorable factors that enhance the risk of cardiovascular diseases and promote carcinogenesis, while LH can contribute to mental retardation and Alzheimer's disease [21, 22, 23].

Thyroid dysfunction affects the reproductive function, causing menstrual irregularities, polycystic ovaries in women, and abnormalities in testicular cell proliferation and differentiation, changes in testicular size, functional and hormonal changes in the male reproductive system [24, 25, 26].

A number of researchers indicate that any dysfunction of the thyroid gland (both hypothyroidism and hyperthyroidism) affects the reproductive system in mammals. In particular, Liu J et al. (2018) found a decrease in the serum GnRH and FSH levels in female rats both in hypothyroidism and hyperthyroidism [27], and Mahmud T et al. (2021) showed that adult rats with hyperthyroidism had low levels of estradiol and progesterone, and the amount of FSH and LH either did not change or decreased [12].

Our study showed that in the female rats with hypothyroidism, an increase in E2 and P4 was determined in the gonads, against the background of a reduced T level, while in the males in the testes, a decrease in the production of testosterone and estradiol, but an increase in progesterone levels were revealed.

We hypothesize that hypothyroidism in cancer, although sharing characteristics with hypothyroidism as
an endocrine disease in its individual appearance, is
a more complex problem due to the involvement of
different regulatory pathways that occur during inter-
actions with malignancy-induced disorders.

The growth of a malignant tumor in experimental
animals influenced the synthesis of sex steroids in the
gonads, while in the females the level of estradiol and
testosterone sharply decreased, but the content of pro-
gesterone increased, whereas in the males the synthe-
sis of all sex steroids was noted in the testes.

In general, it can be said that the tumor process had
a significant impact on the functional activity of the
HPG axis in the animals of both sexes both at the cen-
tral and peripheral levels, but however the recorded
changes in the central regulatory factors differed from
those in hypothyroidism.

The most interesting changes were recorded in the
HPG activity in the main group, where the develop-
ment of the malignant process took place against the
background of hypothyroidism and was inhibited
only in the females. Taking into account the gender
differences in the effect of hypothyroidism on the tu-
mor growth, it turned out that the level of GnRH in
the hypothalamus also changed in different directions,
despite the fact that in the reference groups No. 1 and
No. 2 in the males and females, the content of GnRH
in the hypothalamus changed in the same direction: in
hypothyroidism it decreased, and with the growth of
Guerin's carcinoma the content increased; in the main
group in the females the level of LH-releasing hor-
mone increased, as it was the case with the reference
group No. 2, while in the males it decreased, as it was
the case with the reference group No. 1. That is, the
increase in hypothalamic gonadal releasing has turned
out to be a priority for the females of the main group,
similar to the processes taking place in animals with a
malignant process, while in the males observed was a
decrease, similar to the processes in hypothyroidism.

An interesting fact was that multidirectional changes
in the releasing hormone in the hypothalamus had
the same effect on the level of tropic pituitary hor-
mones, causing an imbalance in the animals of both
sexes towards the prevalence of LH over FSH, due to a
decrease in the follicle-stimulating hormone produc-
tion. Nevertheless, in the gonads of the animals of the
main group, regardless of their gender, a decrease in
the synthesis of the main steroids was noted: it was ap-
picable to estradiol in the females and testosterone in
the males.

In the present study, we showed that hypothy-
roidism affected both the central links of the HPG
axis – the hypothalamus and the pituitary gland, and
the peripheral ones with inhibiting steroidogenesis
in the gonads. In the males, the presence of hypothy-
roidism, both solely and in combination with tumor
growth, initiated secondary hypogonadism, which,
as is known, occurs as a result from abnormalities
of the hypothalamic-pituitary axis, when low levels
of GnRH, LH, or FSH lead to low levels of testoster-
one and spermatogenesis. [28], while in the females,
against the background of disordering of the central
regulatory connections between the hypothalamus
and the pituitary gland, without an ovulatory surge of
gonadotropins in the ovaries, a switch in the produc-
tion of steroids from androgen / estrogen to predom-
nantly progesterone was detected. We assume that a
significant increase in the production of progesterone
by the ovaries of the females of the main group could
contribute to the inhibition of the growth of a malign-
ient tumor.

Thus, it was found that hypothyroidism, as a co-
morbid disease, against which a malignant process de-
velops, has a significant effect both on the central links
of the HPG axis and on the peripheral ones, causing
an additional shift in the hormonal balance of sex ste-
roids.

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