UDC 577.112:577.322:576.342:615.36 https://doi.org/10.15407/biotech14.03.005

CHORIONIC GONADOTROPINE: STRUCTURAL HETEROGENEITY, METABOLIC PATHWAY, FUNCTIONS, OBTAINING AND POSSIBILITIES OF CLINICAL APPLICATION

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Received 01.04.2021
Revised 09.10.2021
Accepted 30.06.2021

Human chorionic gonadotropin (hCG) is one of the key hormones needed for pregnancy sustaining. At the same time, it performs many other biological functions, which is due to the effect on the immune cells' activity, the ability to bind to at least three types of receptors and activate various signaling cascades. Several structural forms of hCG and their combinations have been identified. This structural heterogeneity is the cause of variations not only in the degree and direction of the hormone functional activity, but in the mechanisms of its action, the degree of binding to other molecules and the conditions of dissociation as well.

Aim. To review the current understanding of the role and mechanisms of the biological activity of hCG and its isoforms, as well as the identification of physicochemical factors that affect the completeness of hCG release from biological raw materials and the stability of the isolated drug during further storage.

Methods. A computerized literature search was performed using three electronic databases from 1980 to 2020. Descriptive and comparative analyzes were performed for discovered studies in molecular biology, biochemistry and clinical practice.

Results. A detailed biochemical and physiological analysis of hCG and its related molecules are provided in this review. The features of measuring its content in tissues, isolation and purification methods, difficulties associated with low-temperature storage, as well as the spectrum of hCG preparations clinical use of and their proposed new therapeutic possibilities are considered.

Conclusion. HCG is characterized by a wide range of versatile functions, and its field of application in laboratory diagnostics and clinical practice is still expanding. At the same time, to elucidate the mechanisms of its multiple therapeutic effects, including antitumor action, as well as the mechanisms of dissociation under conditions of low-temperature storage, which can solve the problem of maintaining the stability of this hormone, it remains relevant.

Key words: chorionic gonadotropin; cord blood; α- and β-subunits of hCG; hCG storage.

HCG molecule characteristics and structural features

Human chorionic gonadotropin (hCG) is a member of the glycoprotein hormones family. It is synthesized by placental syncytiotrophoblast cells, as well as syncytial kidneys and free symplasts into the intervillous space, myometrium and blood [1]. HCG can also be produced by neoplasms, in particular, the production of hCG by osteosarcoma cells has been described [2]. In addition, it has been shown that hCG is normally produced in the pituitary gland and eye retina, while it has a local neuroactive effect [3].

This hormone is present in blood serum and urine of pregnant women, along with various dissociated or degraded parts of their

own molecules, which have less pronounced activity or do not possess it. The hCG content in blood and urine increases after embryo implantation into the uterus. The β-subunit of hCG is determined in the mother's blood serum already 8–9 days after conception, Further, the hormone level gradually increases and reaches its maximum by the 5^{th}–7^{th} weeks of pregnancy (5 000–200 000 mIU/ml). From the 8^{th}–15^{th} week of pregnancy, its content decreases (20 000–100 000 mIU/ml) and then remains constant until the 32^{nd}–34^{th} week (15 000–80 000 mIU/ml) [4, 5]. Increased hCG levels are characteristic of multiple pregnancies, while lower levels are characteristic of women of late reproductive age with reproductive pathologies. Literature data analysis shows that the hCG content in the mother's blood differs by orders of magnitude from its level in cord blood, which confirms the presence of separate mechanisms of humoral regulation presence in newborns (Table 1). After childbirth, hCG in the mother's blood ceases to be determined in 1–2 weeks [4].

The researchers associate significant variations in the hCG content in cord blood with high structural heterogeneity of its molecule [8–10]. Thus, serum/urine samples contain many molecules associated with hCG (Table 2).

There are over 100 analytical tests available to quantify hCG. With their help, a full-sized hormone and/or one of seven combinations of other molecules associated with it are detected. The use of antibodies various combinations in commercial test systems leads to the fact that the obtained results can vary significantly. In extreme cases, a 50-fold difference in the determined levels and false-positive/ false-negative results are possible under the condition of normal pregnancy/childbirth. More significant discrepancy can be found at different pregnancy stages or during childbirth with complications [2, 8–10].

Chemically hCG is a glycoprotein with a molecular weight of about 37.5–46.0 kDa, consisting of two subunits alpha and beta, which are non-covalent bonded to each other (Fig. 1).

As it is known, the α-subunit of hCG is identical to the α-subunits of the pituitary hormones and consists of 92 amino acids linked by disulfide bridges, and the β-subunit is specific for hCG. It consists of 145 amino acids. The amino acid sequence of the first 114 amino acids of the β-subunit of hCG is 85% homologous to luteinizing hormone (LH), 36% to follicle stimulating hormone (FS) and 46% to thyroid stimulating hormones (TSH). For example, the β-subunit of LH contains

Table 1. **The hCG content in the serum of cord blood and the fetus mother's blood**

HCG source	HCG concentration in blood serum, IU/L
Mother's Blood [6]	
During pregnancy (end of the third trimester)	2704 ± 18568
In the II stage of labor	2134 ± 28400
Singleton pregnancy (end of the third trimester) [7]	12600 ± 2500
Multiple pregnancy (end of the third trimester) [7]	27500 ± 5000
Cord blood	
According to [6]	8.23 ± 2.57
Singleton pregnancy [7]	130 ± 50
Multiple pregnancy [7]	250 ± 100
Primiparous women [5]	219.2 ± 36.8
Multiparous women [5]	292 ± 41.4

Table 2. **Structural heterogeneity of hCG molecules detected in serum and urine during pregnancy, trophoblastic disease and oncology [adapted from 11]**

HCG molecule	HCG molecule. Molecular weight, kDa
HCG without breaks	37.5
Hyperglycosylated hCG	40
HCG with breaks	36.5
HCG without C-terminal peptide	29
Free β-subunit	22
Free β-subunit with breaks	22
Free α-subunit	14.5
Core fragment of β-subunit	9.5

Note. The table provides a list of hCG molecules recommended by WHO and the International Federation of Clinical Chemistry for pregnancy and trophoblastic tumors determination in laboratory diagnostics.

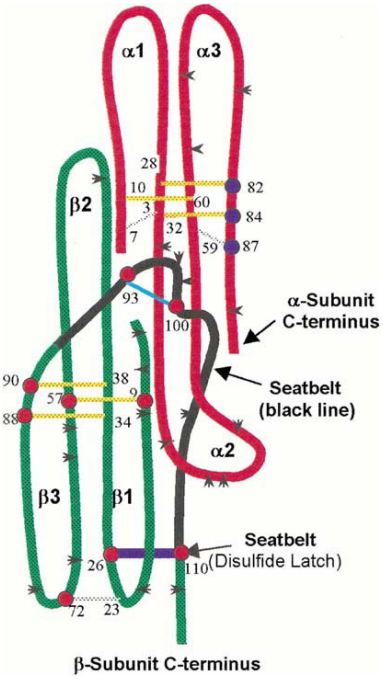

Fig. 1. **Chorionic gonadotropin**
Schematic representation:
α — alpha subunit; β — beta subunit.
Dashes indicate disulfide bridges [12]

121 amino acids, while the β-subunit of hCG contains 145 amino acids. The difference by 24 amino acids between the β-subunits of hCG and LH is unique to hCG and is called the C-terminal peptide [10, 13]. Consequently, some antibodies to hCG recognize also LH, and vice versa [10]. As a result of structural homogeneity, LH and hCG have a common receptor (LH/CG-R), which determines the similarity of their biological properties.

The carbohydrate part of the hormone molecule accounts for about 30% of its molecular weight. The hCG carbohydrate components are necessary for joining subunits, maintaining the conformation of its molecule and protecting polypeptide chains from cleavage by proteolytic enzymes [14]. Removal of carbohydrate residues leads to a significant decrease in the hCG half-life in a body. The combination of two protein chains and 8 side carbohydrate chains leads to high hCG structural heterogeneity.

According to the available information, the hCG synthesis subunits occurs independently in the form of corresponding precursor molecules with a higher molecular weight [2]. The finished subunits are combined into one molecule in the endoplasmic reticulum. The secretion of free hCG subunits occurs either due to the independent regulation of their synthesis, or as a result of their association absence. The fact of α- and β-subunits independent regulation of the synthesis of is proved by the fact that the ratio of their amounts changes during the placenta development. Thus, in the first trimester of pregnancy, the mRNA level of the α-subunit is 2 times higher than that of the β-subunit; at the end of pregnancy, a decrease in the mRNA levels of both subunits is observed in the placenta, but the α: β-ratio increases up to 12:1 [2]. Thus, the regulation of the hCG subunits production is carried out in different ways, but in general, these mechanisms provide the possibility of the biologically active hormone form formation. At the same time, there are data indicating the existence of mechanisms that prevent the hCG dimer formation. One of the ways for disrupting the subunits interaction is hyperglycosylation of the α-subunit, which prevents its participation in the full-sized hCG molecule formation [2].

It is known that the hCG molecule readily dissociates (Fig. 2), and degradation begins with the cleavage of the β-subunit [2]. As a result, the hCG molecule functional activity and stability in blood is sharply reduced [2]. After the β-subunit cleavage, hCG loses its ability to interact with receptors and stimulate progesterone production, and, possibly, acts as the native hormone antagonist of [2].

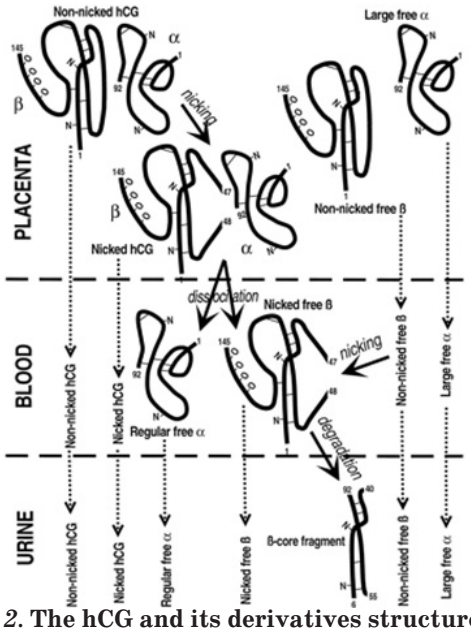

Fig. 2. **The hCG and its derivatives structure [8]**
Black lines correspond to polypeptide chains, the numbers at the lines ends indicate the amino acids number in a chain, thin lines indicate disulfide bonds, the letters N and O indicate the oligosaccharides attachment points, arrows indicate the transition of molecules from placenta to blood and urine, their cleavage and degradation

It is interesting to note that serum free β-subunit levels are relatively low, in average less than 1% of hCG levels at early pregnancy. The content of free α-subunit in blood serum averages 5% of hCG levels at the pregnancy beginning, and at the end of pregnancy its amount increases to 54%. A higher content of free hCG subunits is peculiar to urine samples.

After dissociation, the cleaved β-subunit loses the C-terminal peptide and degrades to the the hCG decay final product — a core-fragment of the β-subunit (Fig. 3).

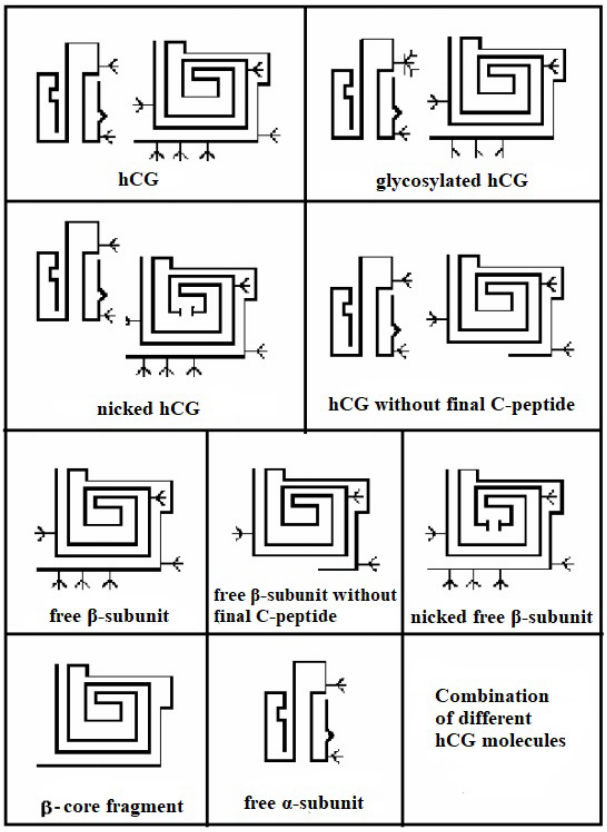

Fig. 3. **Schematic representation of HCG degradation [2]**

The core-fragment of the β-subunit is the main molecule associated with the β-subunit of hCG in pregnant women urine samples. Its concentrations are about 58% of the hCG content in urine at the pregnancy beginning and rise to 305% by the end of pregnancy. This molecule is practically not detected in blood serum (< 0.3% of the hCG concentration).

Thus, hCG has several molecular forms, which, with a common primary structure, differ in their conformation, the location of carbohydrate side chains, the number of binding sites, and, consequently, biological activity [15].

Reception and intracellular signal transduction pathways

The main hCG receptor is LH/hCG-R (85–100 kDa), coupled with G-proteins [16]. Both hCG subunits interact with the receptor, herewith the researchers identify 4 different peptide domains responsible for binding to the receptor with high affinity. The hCG carbohydrate components of are responsible for agonistic activity manifestation because deglycosylated hCG is unable to induce a biological response (increase in cAMP) [17].

It is known that, in addition to LH/hCG-R, some hCG forms are able to interact with the TSH receptor, and glycosylated hCG can also interact with the transforming growth factor β-receptor (TGFβR). A feature of these receptors is the presence of a predominant size ectodomain, which forms a complex site for hCG and other glycoprotein hormones high affinity binding of [18–20]. The result of such binding is a change in the ectodomain conformation and its interaction with other receptor parts. Activation of the transmembrane and cytoplasmic domains and induction of their interaction with different G-proteins types and proteins-regulators of G-protein signaling (RGS-proteins), which superfamily include β-arrestins, mediating the hormonal agents effect on the cascade of mitogen-activated protein kinases and 3-phosphoinositide pathways [20]. Thus, the hCG binding to its receptors leads to the launch of several intracellular signaling cascades at once, which can be realized through various G-proteins types (Fig. 4).

The choice of the dominant signaling pathways after binding to the hormone is determined by active receptor conformations ratio, each of which mediates signal transduction through a specific signaling cascade. This ratio depends on the residence time of the receptor in one or another active conformation, i.e. its relative stability. A decrease in the stability of some active conformations leads to an increase in the proportion of others, which determines the predominant signaling pathways in the cell.

In most cases, natural ligands of receptors associated with G-proteins stabilize not one, but several active conformations, which ratio depends on the heterogeneity of the hormone itself, the receptor microenvironment, features of its structure, as well as on downstream links of signaling cascades availability and functional activity (Fig. 3). Only on rare occasions the natural ligands can activate selectively one specific intracellular cascade.

At the same time, synthetic compounds have been developed that in contrast to natural ligands, activated predominantly one signaling cascade in target cells [21, 22]. The consequence of such compounds selective action of is the lack of a number of functional capabilities appropriate for gonadotropins, including hCG. For example, the compound Org 43553 applications does not lead to ovarian hyperstimulation syndrome, which in the case of gonadotropins is conditioned by the calcium pathway powerful stimulation due to phospholipase C activation [23, 24]. This selectivity can be used in the development of pharmacological preparations for a number of reproductive pathologies correction associated with gonadotropin receptors dysfunction [25] because in this case binding occurs with the transmembrane domain of the receptor, while the extracellular domain remains free. Such compounds are called pharmacoperones (from "pharmacological chaperones") and are currently considered as a promising therapeutic approach to the treatment of disorders provoked by mutations of gonadotropin receptors [25, 26].

HCG non-receptor binding

Currently the question of hCG non-receptor binding and its role is still open and attracts the attention of only a few groups of authors. Although for the first time hCG non-receptor binding was discovered more than three decades ago [27], this topic, apparently, has not been continued and investigated. In [27], the authors observed the hCG adsorption on cell surfaces, including cell membranes from tissues that lack hCG receptors and the hormone itself is not synthesized. So, using specific labeled monoclonal antibodies to hCG, its adsorption was revealed not only by progestational decidual tissues, but also by brain homogenates, fetal lungs and some other tissues. At the same time, the authors emphasize that no such binding with erythrocytes was found. Receptor-bound hCG was not recognized by any of the antibodies used. Thus the authors assumed that the adsorbed hormone has a different orientation compared to the hormone associated with the receptor [27]. The role of non-receptor-coupled hCG has remained uncertain.

In the last decade, the existence of a circulating hCG complex with LH/hCG-R truncated forms, devoid of transmembrane and intracellular domains, has been shown [28–31]. It has been shown that vesicles (50–200 nm) are synthesized in placental tissues, containing several truncated LH/hCG-R isoforms (50, 62 and 75 kDa), a full-sized receptor (85–100 kDa), as well as mRNA, miRNA, growth factors, cytokine and chemokine receptors. It is assumed that these vesicles form a reserve pool of macromolecules for signal transmission over long distances or for the effector ligands adhesion to target cells [32, 33].

Data [28–30] show that microvesicles released by human placenta explants contain both full-sized and LH/hCG-R truncated

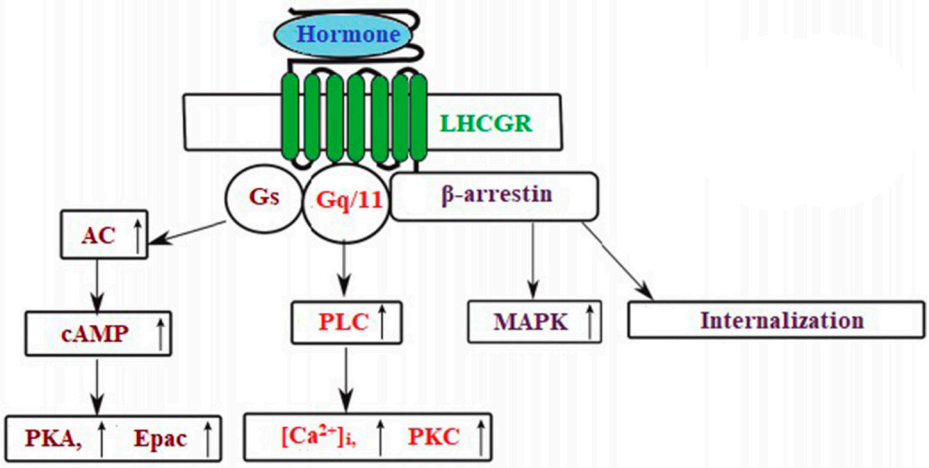

Fig. 4. **Schematic representation of possible activation pathways of intracellular signaling cascades upon hCG binding to LH/hCG-R, functionally coupled with Gs- and Gq/11-proteins and β-arrestin [20]:**
LHCGR — LH and hCG receptor; Gs and Gq/11 are heterotrimeric Gs and Gq/11 proteins; AC — adenylate cyclase; PKA — protein kinase A; Epac — cAMP-dependent exchange factor of the Epac family; PLC — phospholipase C; $[Ca^{2+}]_i$ — concentration of calcium cations inside the cell; PKC — protein kinase C; MAPK — mitogen-activated protein kinases

forms, and these vesicle-associated receptors are able to bind hCG and LH while freely circulating in the blood. Thus, circulating membrane-bound LH/hCG-R can reduce the hormones bioactivity and mediate abnormal tissue activation through the mobile receptors fusion. The authors suggest that microvesicles carrying LH/hCG-R neutralize natural ligands (hCG or LH), forming complexes that prevent the these hormones interaction with tissue-specific receptors. By altering hormone-mediated target cell activation, circulating LH/hCG-R can act as a physiological modulator of hCG and LH function and/or as an inhibitor in hormone dysfunction. Such regulation is more advantageous in terms of speed, since the impact at the level of hormone biosynthesis will lead to a change in its level in the blood only after a few hours [34], while the effect on the already circulating hormone with the help of peptide modulators synthesized "for the future" and stored in microvesicles makes it possible to reduce/increase the active hormone concentration in seconds.

In [30], the analysis of recombinant hCG with LH/hCG-R isoforms complexes was carried out by their separation using polyacrylamide gel electrophoresis under denaturing conditions, and quantitative methods of enzyme-linked immunoassay were developed and tested to determine the levels of serum LH/hCG-R. and hCG-LH/hCG-R complex and to study their correlation with pregnancy outcome. Quantitative analysis of LH/hCG-R and the hCG-LH/hCG-R complex in human serum samples in early pregnancy showed a wide range of circulating receptor concentrations: from 0 to > 3500 pmol/ml [30]. At the same time, a correlation of these indicators with the pregnancy outcome was noticed: pregnancy with an unfavorable outcome was accompanied either by very low (≤5 pmol/ml) or, on the contrary, high (≥170 pmol/ml) concentrations of the circulating hCG complex with the receptor. These observations have enormous diagnostic potential, because serum concentrations monitoring of the truncated LH/hCG-R isoforms and their complexes with hCG can be used to predict a pathological outcome at very early pregnancy stages.

Disruption of the hCG complex with circulating forms of LH/hCG-R formation/dissociation of the could explain the altered ratios of biologically active and immunoreactive hCG and LH, which are often described in patients with hypogonadism, fertility disorders, ovarian dysfunction, recurrent miscarriages and other complications of early pregnancy timing [28]. The possibility that circulating microvesicles carrying LH/hCG-R may fuse with vasculoendothelial cells or some other cell type, thereby making them susceptible to the hCG or LH action, could be another level of complications associated with the disruption of hormonal signals transmission. It is obvious that further detailed study is required to elucidate more precise functional mechanisms, as well as the clinical and diagnostic role of these complexes circulating in blood.

HCG functions

The high hCG heterogeneity, the ability to bind to at least 3 types of receptors (LH/hCG-R, TSH-R and TGFβR) and activate various signaling cascades provide a wide range of its biological functions and various clinical roles, including the diagnosis of pregnancy and its disorders, monitoring of oncological diseases and use as a pharmacological drug.

Each hCG form of is produced by different types of cells and has its own functional profile. For example, hCG and sulfated hCG are synthesized by placental syncytiotrophoblasts and pituitary gland gonadotrophs, respectively. Hyperglycosylated hCG (h-hCG) is produced by placental cytotrophoblasts in the early pregnancy stages and has autocrine effects that reduce myometrial contractility [35] and promote placental angiogenesis [36]. H-hCG practically does not affect the progesterone production, while the endocrine effect of hCG is largely provided precisely by the action on the corpus luteum cells. In addition, h-hCG promotes egg implantation and tumor invasion by acting on cytotrophoblast cells via TGFβR, which has been proven by h-hCG inhibition with specific antibodies [37]. However, the h-hCG functional role and biological activity are still not fully understood and are currently the subject of research [38, 39].

HCG immune functions. Currently, there is some evidence that hCG plays a particularly important role in suppressing immunological responses from the maternal immune system, which prevents ovum rejection [4, 40–42].

The immunosuppressive hCG effects were first demonstrated over 30 years ago by Fuchs et al. in 1980 [43]. This group of authors demonstrated the hCG ability to stimulate lymphocytes that suppress the polyclonal antibodies production by B cells in mice and humans [43, 44]. Later it was shown that the

LH/hCG-R receptor is expressed on T and B lymphocytes [45, 46].

Several studies have examined the mechanism of hCG action on immune cells. In [47], it was found that in female diabetic mice that received recombinant hCG between the 3rd and 15th weeks, the incidence of diabetes significantly decreased, which was associated with a decrease in the proliferative response of T cells against β-cell antigens. In an earlier study, hCG administered to diabetic mice reduced the activation of diabetogenic CD4 and CD8 T cells by activating dendritic cell indoleamine dioxygenase, an effect that was blocked by an inhibitor of the corresponding enzyme [48].

Segerer et al. showed that hCG reduces dendritic cell-mediated activation of autologous T cells [49]. HCG inhibits the dendritic cells proliferation and the production of cytokines [50], and also stimulates the T-suppressors recruitment at the fetus-mother border [42].

It is known that during pregnancy, individual fetal cells, small fragments of chorionic villi and syncytiotrophoblast microparticles enter the mother's bloodstream, which leads to antigenic stimulation and launches a multistep differentiation process of adaptive immune response cells [51]. After contact with a specific antigen, native lymphocytes differentiate into effector cells, and some of them acquire the properties of memory cells [51]. There are few studies investigating the T cells differentiation into memory cells. The group of authors [52] established the hCG role in regulation of the immune memory T cells activity, associated with activation markers CD28 and CD25 expression inhibition on these cells. The hCG depressive effects were observed only at the level of CD^{4+} lymphocytes, without affecting the CD^{8+} subpopulation. Thus, hCG modulates the immune cells functional activity, which can negatively affect the developing embryo, and provide the ratio of various leukocyte subpopulations necessary to maintain immune tolerance.

There is also evidence of the hCG effect on phagocytes. It is known to suppress respiratory burst reactions by triggering the cytochrome P450 degradation [53]. In addition, hCG physiological doses inhibit the activated neutrophils functions, namely the selectins expression, phagocytosis, and the reactive oxygen and nitrogen species production [53]. When studying the hCG effect on the monocytes and eosinophils functions, the dependence of its effects on the concentration, gender and menstrual cycle phase in women was established. Thus, the inhibitory hormone effect on the phagocytic monocytes level in whole blood (phagocytic index), as well as on their absorption activity (phagocytic number) at high doses was revealed [53]. In the case of isolated monocytes of the lutein phase of the cycle, hCG, on the contrary, increased the phagocytic monocytes number and their phagocytic number. The author [53] showed that at the level of peripheral blood monocytes in women, hCG acts through $[Ca^{2+}]_i$, activating a number of enzymes (adenylate cyclase, cyclooxygenase, phospholipase A2). It is possible that in intrauterine infections, when an increased level of pro-inflammatory cytokines is observed, hCG enhances the stimulation of the monocytes phagocytic activity, acting synergistically with cytokines. It is possible that the biological significance of this phenomenon lies in the initiation of miscarriage by gonadotropin in intrauterine infections. Thus, it is obvious that hCG has a pronounced immunomodulatory activity, which direction and severity depend on the hormone dose, the type of target cells and their microenvironment.

The hCG role of in the embryo implantation process. In addition to endocrine effects on the corpus luteum and immune cells, hCG functions as a growth and differentiation factor. According to the literature, it exerts its consistent regulatory action at 3 levels:

• In the early pregnancy stages (before the hCG appearance in the mother's blood), local juxtacrine regulation takes place (the hormone is not secreted, but transported through gap junctions and, reaching neighboring cells, induces an effect in them). It has been shown that the hCG introduction into the women endometrium in the lutein phase of the menstrual cycle (intrauterine microdialysis) has a pronounced effect on the differentiation and implantation parameters [54]. At the same time, an increase in the production of VEGF, a cytokine important for neoangiogenesis, was observed, which suggests an extremely important hCG role in the endometrial vascularization and placentation control. A study of the endometrial remodeling parameters after hCG infusion showed a significant increase in the content of enzymes involved in the remodeling of extracellular matrix structures [54].

• After the appearance in the mother's blood, regulation by hCG acquires a systemic endocrine character. HCG contributes to a very

rapid rise in serum progesterone levels. Other endocrine hCG functions include its thyroid-stimulating activity, as well as modulating the function of the fetal testes, ovaries, and adrenal glands.

• The third level of hCG regulation — autocrine — becomes possible after the expression of full-sized LH/hCG-R on the trophoblasts themselves. Up to the 9th week of pregnancy, human villous trophoblasts express a truncated hCG/LH-R isoform (50 kDa) (which has recently been shown to bind free hCG with the circulating complex formation [55]). Later, the full-sized receptor (80 kDa) begins to be expressed, which allows hCG to modulate the differentiation of the trophoblasts themselves. A feature is the self-regulation of hCG synthesis, which can partially explain the unique profile of hormone secretion with a peak level during I trimester and its rapid decline after the 10th week of pregnancy.

HCG role of in the pathogenesis of autoimmune diseases. It is known that in autoimmune thyroiditis, there is an increased frequency of reproductive disorders, recurrent miscarriages and infertility in women and laboratory animals [56–58]. Although the exact mechanism of this association is unclear, it is likely that it is associated with T-suppressors dysfunction, which provokes both autoimmune reactions and recurrent miscarriages [59].

The mother's thyroid function is critical to the embryo development, especially early in pregnancy. Since hCG has a TSH-like effect, high concentrations of it lead to thyroid stimulation, both functionally (lower serum TSH concentrations) and anatomically (increased thyroid volume).

At the same time, it has been established that in such cases the presence of maternal autoantibodies to hCG is often determined, which interfere with its full functioning. Thus, in the works [60, 61] it was shown that a quarter of surveyed women with primary or secondary infertility had antibodies to hCG in their serum. It is interesting that, these indicators were comparable to those in women with antiphospholipid and antitrophoblastic antibodies.

Due to the of hCG and TSH molecular similarity, it is assumed that there are autoantibodies to LH/hCG-R, which, as in the case of autoimmunity to TSH receptors, provoke the hyperthyroidism development and an increased miscarriages frequency. That is, if TSH can activate LH/hCG-R, then autoantibodies that block the TSH receptor can also block LH/hCG-R in the corpus luteum through similar cross-reactivity [62]. It should be noted that in the late 80s antibodies to LH/hCG-R were observed in women with primary and secondary infertility. They were detected using the ELISA method and LH/hCG-R, isolated using affinity chromatography from the corpus luteum of cattle [63], however, apparently, this work has not received proper distribution.

Thus, we can conclude that hCG performs many local and systemic functions inside and outside the embryo-endometrial microenvironment, which determines its exceptional role in understanding and correcting a wide range of reproductive, immune and endocrine disorders.

HCG determination, isolation and purification

Test systems for the hCG and its derivatives determination. Many technologies have been developed to determine the hCG concentration, including electrochemical, chemiluminescence and fluorescence immunoassays, resonance scattering spectrometry, atomic emission spectrometry, radio immunoassays, and so on. Some tests focus on simplicity and ease of use, while others focus on accuracy and application in clinical medicine.

To date, the quantitative hCG determination is carried out mainly using different types of immunoassay using monoclonal and/or polyclonal antibodies [64, 65]. Different hCG molecules have multiple binding sites to which the corresponding antibodies have been developed. Five different binding sites for antibodies were identified on the uncleaved hCG, 4 binding sites on the cleaved hCG, 2 on the free β (or α)-subunit, 6 on the uncleaved free β-subunit, 5 on the cleaved free β-subunit, and 4 sites on the core -fragment of the β-subunit, as well as 4 epitopes, which are formed as a result of the fusion of 2 subunits [2, 17, 66]. In all tests for hCG determination, there is at least 1 antibody to the β-subunit, therefore the term β-hCG is usually used for any methods for hCG or β-hCG determination. However, epitopes are detected on free β-hCG, antibodies to which do not interact with the full-sized hCG molecule [2, 66], and, therefore, when they are used, differences in the results may occur, which was discussed above. Most commercial kits for hCG determination include several antibodies targeting different binding sites of the hCG

molecule and its isoforms. Some define only uncleaved hCG, others uncleaved hCG and a free β-subunit, and still others — a cleaved and uncleaved hCG and a free β-subunit. There are a limited number of systems that define all types of molecules, including the core-fragment of the β-subunit. As a result of such variety of antibodies used, commercial hCG test systems can measure a fairly wide range of associated molecules.

HCG Isolation and purification. For modern pharmaceuticals production, 2 types of hCG are used — of natural origin, extracted from the urine of pregnant women — u-hCG, and a hormone obtained using recombinant technologies — r-hCG [67]. Attempts to purify hCG from urine of pregnant women and patients with trophoblastic diseases have been carried out since the hCG discovery in 1927 [68]. The first commercial drug was the hCG extract released by the Organon company in 1931 [69].

Most of the protocols for hCG obtaining, their advantages and disadvantages are described in detail in an earlier work [70]. After a while, the procedures for hCG isolation and purification have been improved [68]. For these purposes, several sequential techniques are mainly used, including absorption, gel exclusion chromatography, fractionation on ion exchange columns or columns with concanavalin A and sepharose. Using the described extraction and fractionation methods by the authors of [68], 5.11 g of acetone precipitate with a protein content of 2.89 g was obtained from 33.0 liters of combined urine.

However, it is generally known that urine samples contain different hCG forms, including degraded and hyperglycosylated molecules, free subunits and their fragments, which have little or no biological activity. In addition, it has been shown that u-hCG preparations additionally contain impurity proteins (proteolytic enzymes, early pregnancy factor (EPF) 10, epidermal growth factor) and pollutants inhibiting gonadotropin [67, 70, 72]. The relative amount of impurity proteins and hCG significantly differs in different batches [67, 71]. Thus, in order to obtain highly purified u-hCG preparations, the process of its isolation and purification needs to be improved.

In this regard, the technology for r-hCG production was introduced, which provided greater purity and high reproducibility from batch to batch, as well as the control improving possibility of the active substance final concentration. However, some independent studies indicate that r-hCG preparations contain traces of contaminating proteins as well that are not listed in the manufacturer's instructions [67].

It should also be noted that back in the mid-90s the works were actively carried out on synthetic hCG fragments creation, for which various methods and approaches were used such as the hybrid molecules construction, directed mutagenesis, proteolytic fragmentation, chemical modification of molecules. This led to the new compounds creation with a narrower spectrum of biological activity than hCG and which were potential drugs for clinical use [73–75]. However, despite developments in the field of synthetic compounds creation and the r-hCG, urine-derived preparations are still widely used due to their low cost and availability, not only in low-income countries, but also in Europe and the United States [67, 76]. In addition, the demand for these drugs is growing due to demographic factors such as an aging population and an increase in the average age of motherhood. Therefore, the search for alternative or additional sources of raw materials for hCG production remains an urgent issue, which solution would help to solve partially the described problems. One of such sources may be human cord blood.

It should also be noted that information about the terms and storage conditions of an isolated hCG preparation is very contradictory. The problem of this hormone stability is important not only for determining the conditions of use, transportation and storage of pharmaceutical preparations based on it. This issue is of great importance in clinical laboratory practice in the hCG content quantitative measurement of the in biological material and the test system calibration using lyophilized standards. Most hCG test kit instructions for the quantitative hCG determination indicate that reconstituted standards are stable for 2 months at 2 °C to 8 °C. For longer storage, it is recommended to store them at −20 °C. At the same time, there are experimental data indicating a significant loss of intact hCG immunoreactivity (in the range of 30–40%) when stored at −20 °C [77]. Other authors, on the contrary, showed an increase in the hCG immunoreactivity, which was expressed in an increase in its concentration by 52% and 25% from the initial level for 4 weeks of storage at 4 °C and −20 °C, respectively [78]. In [79, 80], the stability of intact hCG and β-hCG was assessed under storage conditions at 21 °C,

4 °C, and −20 °C, while no changes in their immunoactivity were found under most storage conditions. However, in a study of the disrupted hCG and free β-subunits levels after 4 weeks of blood serum storage at 4 °C showed an increase in these indicators to 360 ± 53% of the initial level [81]. Most authors suggest that the reason for changes in the hCG content in samples with the course of storage time may be the appearance of hCG with breaks and its dissociation that is confirmed by a parallel increase in the free β-subunit concentration. In addition, due to the unique characteristics of each antigen-antibody binding site, the post-storage hCG levels obtained may differ when using different immunoassay test systems.

Thus, maintaining the hCG stability in biological samples, standards for test systems and pharmaceuticals during storage is an urgent problem. A detailed study of the hCG degradation mechanisms under conditions of low-temperature storage is necessary in order to minimize its dissociation and errors in analysis, which is critically important for clinical practice.

Clinical application

Because of its unique properties, hCG has a broad therapeutic profile. Currently, in clinical practice, hCG preparations are often and almost uncontestedly used to correct the reproductive system disorders and in assisted reproductive technologies [20, 51, 82]. Partially purified u-hCG preparations are used as a substitute for LH to achieve final oocyte maturation and ovulation during controlled ovarian hyperstimulation and to facilitate the correct timing of oocyte retrieval in IVF cycles [83, 84]. In addition, it has been shown that the hCG administration to recipients on the day of embryo transfer increases the endometrium thickness and improves its susceptibility [85]. However, recent studies have shown that when they bind to LH/hCG-R, LH and hCG each trigger different intracellular signaling cascades (AKT, ERK1/2 MAPK, and β-arrestin 2) and steroidogenesis [86, 87]. Similarly, the hCG use in IVF may lead to different responses compared to LH in terms of mature oocyte collection, embryo quality, implantation, and pregnancy rates [88, 89].

Over the last years, studies have been conducted on the hCG use effectiveness in men's pathospermia, while spermatogenesis stimulation and an increase in fertility have been noted [90–93]. In most studies, hCG monotherapy led to an increase in the total spermatozoa number, their motility, and in some cases contributed to an increase in the percentage of morphologically normal sperm forms. However, in regard to the oligoteratozoospermia elimination and the spontaneous conception achievement, the combination therapy of hCG and FSH turned out to be the most effective for men [94].

The researchers have discovered as well the hCG ability to influence the inflammation development: the β-hCG (or synthetic analogs) introduction of in critical conditions induced by lipopolysaccharides stops inflammatory reactions [95, 96]. In addition, some β-hCG-like oligopeptides can block not only inflammation, but also septic shock, type I diabetes outbreak, renal failure, tumor growth, and neutralize the radiation sickness effects [97–99]. These results open up prospects for new therapeutic agents creation. Currently, a number of studies are being carried out on the safety, tolerability, pharmacokinetics and pharmacodynamics of such drugs in various systemic diseases associated with inflammation [99–101].

Another promising direction for research is associated with the hCG effect on the proliferation and apoptosis of malignant transformed cells. In the works of various authors, the hormone inhibited the tumor cells multiplication in Kaposi's sarcoma, squamous cell lung cancer, prostate cancer, acute myeloid leukemia [102–104], but stimulated the tumor cells growth in epithelial carcinomas such as cervical carcinoma and choriocarcinoma [102]. An array of conflicting data has also been accumulated regarding the hCG effect on the breast cancer development, while most researchers have confirmed the suppressive effects of the hormone or its partial analogs on cancer cells of this type [103–107].

From the data presented, it is obvious that hCG preparations are capable of exerting the opposite effect on the oncological diseases development. The reasons for this are still unclear and are being discussed by researchers. One of the reasons may be a high degree of hCG heterogeneity, another — a large number of LH/hCG-R polymorphisms, which causes different receptor activity. In addition, the genes encoding hCG have also many polymorphisms, as a result of which different hormone molecules types can be expressed. Nevertheless, the conflicting the hCG effects in oncology have led to the hypothesis that the use of its drugs may become a way to prevent cancer and/or a new immunological approach to its therapy.

Currently, the information about the hCG functional, therapeutic role of and the possibilities of its use in the clinic continues are going to replenish.

Conclusions

Summarizing the literature data presented in this review, we can come to the following conclusions: to date, it has been possible to identify all levels of the hCG structural organization of and, on the basis of these data, to synthesize a biologically active analogue of this hormone and its peptide fragments. It has been possible also to establish that hCG degradation products have some biological activity, which is extremely important for understanding the mechanism of its action. It has been enabled to make significant progress in understanding the process of hCG interaction with cellular receptors. A number of methods have been developed for the hCG and its fragments quantitative determination of. Various biotechnological methods of hCG industrial production have been created and the basic principles of this hormone low-temperature storage in a liquid and lyophilized state at various temperatures have been determined. The possibilities of its therapeutic use in the reproductive system diseases in both women and men have been established.

However, despite the above achievements, the questions about the hCG role in the autoimmune diseases pathogenesis and the mechanisms of its multiple therapeutic effects, including antitumor action require further clarification. The process of isolating and purifying hCG from natural sources also needs to be improved. In addition, in view of the conflicting data on the isolated hCG preparation storage terms and conditions, a more detailed study of its dissociation mechanisms under conditions of low-temperature storage is necessary, which can solve the problem of this hormone stability maintaining.

The review was prepared within the framework of the scientific work "Study of the regularities of the release of biologically active water-soluble substances release from tissues of plant and animal origin when exposed to low temperatures" (State registration number 0106V002165, execution period 2020–2024), which is funded from the budget of the National Academy of Sciences of Ukraine.

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ХОРІОНІЧНИЙ ГОНАДОТРОПІН: СТРУКТУРНА ГЕТЕРОГЕННІСТЬ, МЕТАБОЛІЧНИЙ ШЛЯХ, ФУНКЦІЇ, ОТРИМАННЯ ТА МОЖЛИВОСТІ КЛІНІЧНОГО ЗАСТОСУВАННЯ

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Хоріонічний гонадотропін людини (ХГЛ) є одним із ключових гормонів, необхідних для підтримання вагітності. Водночас він виконує багато інших біологічних функцій, що зумовлено впливом на активність імунних клітин, здатністю взаємодіяти як мінімум з трьома типами рецепторів і активувати різні сигнальні каскади. Було ідентифіковано кілька структурних форм ХГЛ і їх комбінацій. Така структурна гетерогенність є причиною варіацій не тільки виявленості та спрямованості функціональної активності гормону, але й механізмів його дії, ступеня зв'язування з іншими молекулами і умов дисоціації.

Мета. Огляд сучасного уявлення про роль і механізми біологічної активності ХГЛ та його ізоформ, а також виявлення фізико-хімічних чинників, що впливають на повноту вивільнення ХГЛ з біологічної сировини і стабільність ізольованого препарату при зберіганні.

Методи. Проведено комп'ютеризований пошук літератури з використанням трьох електронних баз даних із 1980 до 2020 року. Для виявлених досліджень з молекулярної біології, біохімії та клінічної практики проведено описовий та порівняльний аналіз.

Результати. В огляді подано детальний біохімічний і фізіологічний аналіз ХГЛ і його споріднених молекул. Розглянуто особливості вимірювання його вмісту в тканинах, методи виділення і очищення, складнощі, пов'язані з низькотемпературним зберіганням, а також спектр клінічного застосування препаратів ХГЛ і передбачувані на сьогодні їхні нові терапевтичні можливості.

Висновки. ХГЛ характеризується великим спектром різнопланових функцій, і сфера його застосування в лабораторній діагностиці та клінічній практиці дедалі розширюється. У той же час залишається актуальним з'ясувати механізми його множинних терапевтичних ефектів, у тому числі протипухлинної дії, а також механізми дисоціації за умов низькотемпературного зберігання, що може вирішити проблему підтримання стабільності цього гормону.

Ключові слова: хоріонічний гонадотропін; кордова кров; α- і β-субодиниці ХГЛ; зберігання ХГЛ.

ХОРИОНИЧЕСКИЙ ГОНАДОТРОПИН: СТРУКТУРНАЯ ГЕТЕРОГЕННОСТЬ, МЕТАБОЛИЧЕСКИЙ ПУТЬ, ФУНКЦИИ, ПОЛУЧЕНИЕ И ВОЗМОЖНОСТИ КЛИНИЧЕСКОГО ПРИМЕНЕНИЯ

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Хорионический гонадотропин человека (ХГЧ) представляет собой один из ключевых гормонов, необходимых для поддержания беременности. В то же время он выполняет множество других биологических функций, что обусловлено влиянием на активность иммунных клеток, способностью связываться как минимум с тремя типами рецепторов и активировать различные сигнальные каскады. Было идентифицировано несколько структурных форм ХГЧ и их комбинаций. Такая структурная гетерогенность является причиной вариаций не только выраженности и направленности функциональной активности гормона, но и механизмов его действия, степени связывания с другими молекулами и условий диссоциации.

Цель. Обзор современного представления о роли и механизмах биологической активности ХГЧ и его изоформ, а также выявление физико-химических факторов, влияющих на полноту высвобождения ХГЧ из биологического сырья и стабильность изолированного препарата при хранении.

Методы. Проведен компьютеризированный поиск литературы с использованием трех электронных баз данных с 1980 по 2020 год. Для обнаруженных исследований по молекулярной биологии, биохимии и клинической практике проведен описательный и сравнительный анализ.

Результаты. В обзоре представлен подробный биохимический и физиологический анализ ХГЧ и его родственных молекул. Рассмотрены особенности измерения его содержания в тканях, методы выделения и очистки, сложности, связанные с низкотемпературным хранением, а также спектр клинического применения препаратов ХГЧ и предполагаемые на сегодняшний день их новые терапевтические возможности.

Выводы. ХГЧ характеризуется большим спектром разноплановых функций, и область его применения в лабораторной диагностике и клинической практике все еще расширяется. В то же время остается актуальным выяснение механизмов его множественных терапевтических эффектов, в том числе противоопухолевого действия, а также механизмов диссоциации в условиях низкотемпературного хранения, что может решить проблему поддержания стабильности этого гормона.

Ключевые слова: хорионический гонадотропин; кордовая кровь; α- и β-субъединицы ХГЧ; хранение ХГЧ.