EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Phosphorylation and Carbonylation of Placental Proteins in Normal Pregnancy and Gestosis

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Study of posttranslational changes in placental proteins revealed disorders in the intensity of their phosphorylation and carbonylation in patients with placental failure. Phosphorylation was reduced for the majority of endogenous placental proteins, substrates for cAMP- and cGMP-dependent protein kinases. An opposite dynamics was noted for oxidative modification of proteins. The content of carbonyl derivatives evaluated in spontaneous and metal-catalyzed oxidation of placental proteins was elevated in gestosis in comparison with the normal level.

Key Words: *cAMP-dependent protein kinases; cGMP-dependent protein kinases; protein phosphorylation; protein carbonylation; placenta*

Functional integrity of the placenta providing normal relationship between maternal and fetal organisms is largely determined by the status of the protein component of this important organ of the female reproductive system. Proteins performing numerous biological functions serve a "molecular machine" realizing information program of the cells. Changes in the structure, physicochemical characteristics, or enzymatic activity of placental proteins can become a cause of placental failure (PF) associated with many complications of gestation. Posttranslational modification of protein molecules (including their phosphorylation and carbonylation) plays an important role in the regulation of cellular processes. According to modern notions, protein phosphorylation under the effects of cAMP- and cGMP-dependent protein kinases is the main mechanism realizing the biological effects of cAMP and cGMP. Phosphorylation reactions are involved in interaction of many hormones with the corresponding receptors, this, in turn, providing adequate hormonal regulation. Hormone regulation of chemical composition and functions is of crucial importance in the placenta devoid of innervation. Hormonal imbalance in the mother—placenta—fetus system is an important factor complicating gestation and development of the fetus [4]. Among these factors are also disorders in free-radical processes, which are now regarded as a universal mechanism involved in the development of various complications of pregnancy, including PF usually paralleled by hypoxia. Proteins, whose oxidative forms serve as early markers of cell damage, can be substrates of free-radical modification in intrauterine hypoxia. One of the main reactions of oxidative modification of proteins is their carbonylation [5]. Since many proteins with enzyme and hormonal activities are subjected to carbonylation and phosphorylation, the spectrum of cell functions regulated by these processes is very wide.

The significance of these types of protein modification and the absence of data on the dynamics of these processes in the placenta during gestation

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prompted us to study phosphorylation and carbonylation of placental proteins during normal pregnancy and in gestosis complicated by PF.

MATERIALS AND METHODS

Placentas from 65 women aged 22-29 years were examined. Group 1 consisted of 25 women with PF who had full-term delivery (39-40 weeks), group 2 consisted of 20 women with PF with preterm delivery at 36-37 weeks, and control group consisted of 20 women with uneventful pregnancy and normal labor. Placental failure was diagnosed after comprehensive dynamic studies (evaluation of hormonal status, ultrasonic and dopplerometric studies, and measurements of specific placental isoenzymes alkaline phosphatase and glutamate dehydrogenase) [3].

The nuclei, mitochondria, and cytoplasm were isolated from the placental tissue by standard differential centrifugation in sucrose density gradient (supernatant after centrifugation at 105,000*g*). The purity of fractions was evaluated microscopically. Non-histone proteins of the chromatin [7] were isolated from the nuclear fraction after iso-osmotic lysis, extraction, and differential centrifugation; these proteins served as the endogenous substrates for placental protein kinases. Summary cytoplasmic and mitochondrial proteins were also used for this purpose. Placental extracts (10%) served as the enzyme sources. Activity of cyclonucleotide-dependent protein kinases was evaluated by incorporation of radioactive phosphorus into proteins from [γ^{-32}P] ATP. The incubation mixture contained 10 mM K-phosphate buffer (pH 7.4), 1 mM $MgCl_2$, 1 mM dithiotreitol (Reanal), 0.2 μCi $^{-32}$P-ATP (Amersham), and 10 μM cAMP (Sigma) for detection of cAMP-dependent protein kinase (cAMP-PK) and 10 μM cGMP (Sigma) for detection of cGMP-dependent protein kinase (cGMP-PK). The preparations were incubated for 20 min at 37°C. After reaction, the contents of the tubes was transferred to Synpor filters with 0.4-μ pores, washed with stop-solution, dried, and radioactivity was measured in a scintillation counter.

Protein carbonylation in the isolated subcellular fractions was evaluated by the reaction of oxidized amino acid residues (carbonyl derivatives) with 2,4-dinitrophenylhydrasine (2-4-DNPH) with the formation of 2,4-dinitrophenylhydrasones, which were detected spectrophotometrically [1]. Spontaneous and metal-catalyzed protein oxidation was evaluated as follows. Protein oxidation was initiated by incubation of placental extracts with bivalent iron salts and hydrogen peroxide solutions. The results were expressed per gram protein.

The data were statistically processed using Statistica 5.1 software. The significance of differences between the parameters was evaluated by Student's test and its analog for non-parametric distribution (Mann—Whitney test). The results were significant at $p<0.05$.

RESULTS

Our studies showed that the intensity of placental protein phosphorylation depended on the cyclic nucleotide regulating protein kinase activity. In normal pregnancy, cAMP-PK activity surpassed cGMP-PK activity for the substrate proteins of the placental nuclear and mitochondrial fractions, but was below cGMP-PK activity for phosphorylation of cytoplasmic protein (Table 1). In pregnancy complicated with PF and eventuating in full-term delivery (39-40 weeks), activity of cAMP-PK decreased to a different measure for all studied substrates. This activity dropped most markedly (by 38%) for mitochondrial proteins. Phosphorylation of non-histone proteins of chromatin and cytoplasmic proteins catalyzed by cAMP-PK decreased by 31 and 22% compared to the corresponding parameters in normal gestation. Activity of placental cGMP-PK was also 20-22% reduced for nuclear and cytoplasmic proteins as substrates under conditions of PF. On the other hand, cGMP-dependent mitochondrial protein phosphorylation was 25.6% higher than in normally developing placenta.

In women with pregnancy eventuating in preterm delivery (36-37 weeks) under conditions of PF, disorders in placental protein phosphorylation were more pronounced. Activity of cAMP-PK during phosphorylation of non-histone proteins of chromatin and cytoplasmic proteins in these patients decreased by 43.5 and 30%, respectively, compared to women with normal gestation. The intensity of cAMP-dependent phosphorylation of placental mitochondrial proteins in this group was almost 2-fold below the normal. In contrast to cAMP-PK activity changing in the same direction in full-term and preterm delivery under conditions of PF, mitochondrial protein phosphorylation under the effect of cGMP-PK was elevated in group 1 women and reduced in women with preterm delivery. The increase cGMP-dependent phosphorylation of placental mitochondrial proteins in women with full-term delivery was presumably compensatory and directed to the maintenance of cell respiration under conditions of hypoxia in PF.

One of the main mechanisms in the common chain of metabolic breaks in the placenta forming against the background of intrauterine hypoxia is

TABLE 1. Activities of cAMP- and cGMP-Dependent Protein Kinases (cpm/mg protein) in the Placenta in Normal Gestation and PF (*M*±*m*)

Substrate proteins	cAMP-PK activity			cGMP-PK activity		
	control	group 1	group 2	control	group 1	group 2
Chromatin non-histone proteins	54.32±4.61	37.56±3.13**	30.77±2.74**	37.65±2.82	29.96±1.64*	26.37±2.41**
Mitochondrial proteins	29.64±2.10	18.33±1.52**	15.26±1.41***	20.34±1.73	25.55±1.94*	15.92±1.21*
Cytoplasmic proteins	38.54±2.71	30.16±2.34**	27.13±1.80**	47.25±3.22	37.13±2.52*	32.34±2.91**

Note. Here and in Table 2: *p<0.05, **p<0.01, ***p<0.001 compared to the control.

TABLE 2. Activities of Carbonyl Derivatives (μmol/g) in the Placenta during Normal Gestation and in PF (*M*±*m*)

Substrate proteins	Spontaneous oxidation of proteins			Metal-catalyzed oxidation of proteins		
	control	group 1	group 2	control	group 1	group 2
Chromatin non-histone proteins	1.62±0.08	1.98±0.09*	2.11±0.14*	1.95±0.09	2.55±0.15**	2.68±0.14***
Mitochondrial proteins	3.49±0.16	4.79±0.25**	5.28±0.36*	4.63±0.24	6.78±0.42***	7.54±0.51***
Cytoplasmic proteins	2.31±0.12	2.97±0.21**	3.27±0.20***	2.89±0.18	4.06±0.25**	4.28±0.29***

free-radical damage to cell components because of hyperproduction of active oxygen forms induced by the pro-/antioxidant imbalance. Our studies showed that PF led to more intense oxidative modification of proteins of all studied placental subcellular fractions (Table 2). If complicated pregnancy eventuated in full-term delivery, both variants of oxidative modification of proteins (spontaneous and metal-catalyzed) were maximally increased in the mitochondrial fraction (by 37.2 and 46.4%, respectively). The least increment in the content of carbonyl derivatives was detected for nonhistone chromatin proteins. The intensity of free radical oxidation of this fraction in PF was 22.2% higher than normally and of metal-catalyzed oxidation was by 30.7% above the physiological level. Spontaneous oxidation of cytoplasmic proteins increased by 28.5%. Carbonylation of placental cytoplasmic proteins in the presence of electron donors and metal of alternating valency (Fe^{2+}) was 40.4% higher in group 1 women than in controls. The increment in the content of carbonyl derivatives of free and metal-catalyzed oxidation of proteins was more pronounced in the placentas of women with preterm delivery under conditions of PF in comparison with full-term delivery. Oxidative modification of proteins in this group of women was the highest in the mitochondrial fraction (similarly as in group 1). The content of carbonyl derivatives in the mitochondrial proteins in preterm delivery was more than 1.5 times higher than in normal delivery, presumably because of hypersensitivity of placental mitochondrial proteins to free radicals under conditions of intrauterine hypoxia [8]. Intensification of protein carbonylation in the nuclear and cytoplasmic fractions varied from 30 to 45%, depending on the type of oxidative modification.

The detected intensification of oxidative modification of proteins in different placental subcellular fractions, particularly of metal-catalyzed oxidation, evaluated by the level of carbonyl derivatives, can be paralleled by disorders in protein structure at different levels under conditions of PF [6]. Free radicals, for example, hydroxyl radical and superoxide anion radical, can destroy not only lateral chains of amino acid residues, but can also oxidize the protein molecule skeleton in the α-carbon atom region and hence, modify its function [2]. The detected modification of placental protein phosphorylation can also significantly modulate the biochemical mechanisms of cell regulation. Even minor changes in the phosphorylation degree of placental cytoplasmic proteins (almost $^1/_3$ of the protein stock of the placenta), including the specific pregnancy proteins and performing numerous functions, promote the development of deep changes in the placenta. This is also true for the mitochondrial proteins responsible for the energy potential of the cells. Reduction of phosphorylation of chromatin non-histone proteins, regulating the DNA biological activity and serving as acceptors for steroid receptor complexes, is particularly hazardous for the metabolic processes in the placenta.

Hence, PF is associated with posttranslational modification of placental proteins (phosphorylation and carbonylation disorders), which play an im-

portant role in the mechanisms of this complication of gestation, leading, in turn, to deterioration of metabolism in the mother—fetus system in general.

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