Composition of Protein-Peptide Complex of Human Fetal and Rabbit Juvenile Tissues

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The composition of the protein-peptide complex of cryopreserved human fetal tissues and juvenile tissues of newborn rabbits was studied by spectrophotometric and chromatographic methods (liquid, thin layer, and reaction paper chromatography).

Key Words: *low- and medium-weight molecular peptides; chromatography; fetal tissues; juvenile tissues*

The use of fetal cells and tissues in biology and medicine attracts much interest now. Preliminary results of their use for the treatment of different pathologies of the CNS, endocrine organs, liver, hemopoietic organs in malignant neoplasms, and atherosclerosis leave no doubt in good prospects of this trend [2,5,8,9]. However, the composition of fetal tissues and mechanisms underlying their effect on the organism remain poorly studied.

Here we studied low- and medium-molecular-weight substances and their peptide component in human fetal and rabbit juvenile tissues.

MATERIALS AND METHODS

Protein-peptide extracts were prepared from human fetal tissues (18-20-week gestation) and rabbit juvenile tissues (days 1-2 of neonatal period). Supernatants of acetic acid suspensions of the studied samples were analyzed. High-molecular-weight proteins were precipitated with acetone (1:10 v/v) for 15-20 h on the cold, free amino acids and biogenic amines remained in the supernatant. The peptide fraction was isolated by treatment of the precipitate by 0.1 M acetic acid followed by lyophilization [6,7].

The presence of compounds with different chemical structure, products of normal metabolism, was confirmed by spectrophotometry on an SF-46 at λ=210-300 nm [3]. The groups of compounds were identified by dominant extinctions of peptide chromophores and other nitrogen-containing substances. More fundamental identification of components was carried out by reverse-phase liquid chromatography on a Milichrom chromatograph under conditions for peptide separation. The method detects peptides in concentrations of 1-10 nM [6,7]. Thin-layer chromatography on highly effective cation- and anion-exchange resins and on silica gel with buffer eluents (pH 4) [6,8] identified amino acids and peptides in fractions with complex composition. Hydrolysates of peptide fractions were studied under the same conditions for identification of the structure of individual peptides [1]. The predominant content of bioactive metabolites was detected for each case by express method of paper chromatography [4].

RESULTS

Cryopreserved fetal tissues most often used in clinical practice were analyzed: heart, liver, lung, kidney, and placenta. Spectrogram of human fetal tissue is presented by a curve with two peaks (Fig. 1) at 210-220 and 270-280 nm. The shape of the curve indicates that fetal tissues contain chemical compounds of different classes and are similar by their qualitative characteristics.

The peptide component of all fetal tissues is presented mainly by medium- and low-molecular-weight

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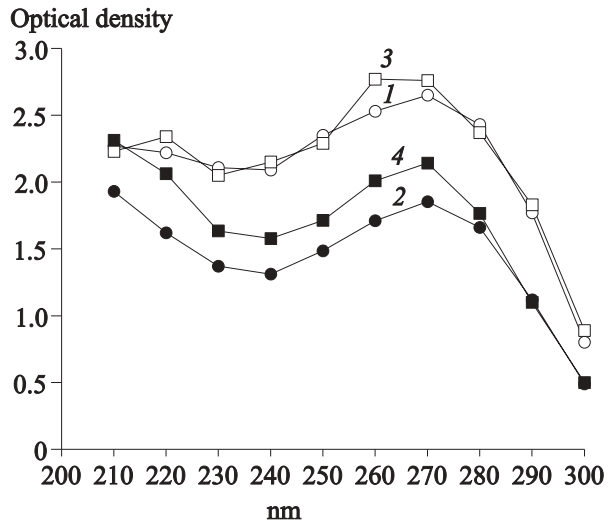

Fig. 1. Spectrograms of human fetal tissues. *1*) heart; *2*) liver; *3*) lung; *4*) placenta.

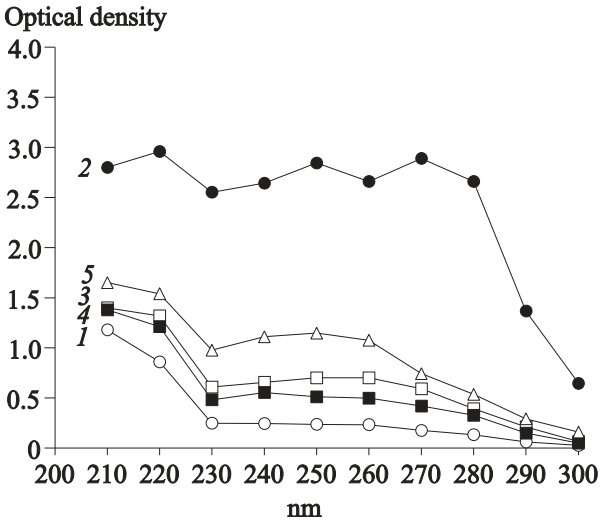

Fig. 2. Spectrograms of rabbit juvenile tissues. *1*) heart; *2*) liver; *3*) lung; *4*) kidney; *5*) brain.

fractions with predominating short-chain peptides (molecular weight <1 kDa) with sulfide bridges. Met- and leu-enkephalins, angiotensins, kinin derivatives (kallidin, bradykinin), substance P, somatostatin, gastrin and taftsin fragments were identified in the peptide extracts. The peptide components of fetal tissues differed by not only qualitative, but also quantitative content of individual fractions. The placenta was characterized by the highest content of peptides. Angiotensin compounds were present in all tissues. Peptides belonging to the taftsin family predominated in the heart. Substance P and related peptides were identified in all fetal tissues. Terminal amino acids Asn, His, Glu, and Asp, the most active and participating in many biochemical transformations and physiological processes, predominated in fetal tissue peptides. Fetal tissues contained a wide spectrum of free amino acids, their content was maximum in the lungs. The contents of free amino acids in the liver, heart, and placenta were similar.

The system of sulfur-containing amino acids and their metabolites is well presented in all studied fetal tissues: glutathione, methionine, cysteine, homocysteine, homocysteic acid participating in redox reactions. The content of sulfur-containing components is maximum in the placenta and lower in the lung, heart, and liver. In fetal material the amino acid component is better presented than the peptide component. We also studied juvenile rabbit tissues.

Extinction of the peptide chromophore 210-220 nm predominated in juvenile tissues (Fig. 2), especially in the liver and brain, as well as in the fraction including fragments of nucleic acids. Chromatographic methods showed the presence of short-chain peptides with molecular weights of 1.0, 1.2, and 1.5 kDa in all tissues.

The heart contained primarily peptides with a molecular weight below 1 kDa, the liver 1.0-1.2 kDa, and the brain 1.5 kDa. Comparative computer analysis showed that the content and spectrum of peptides decreased in the following order: brain, heart, lung, liver, and kidneys. Rabbit tissues contained nitrogen-containing fragments of nucleic acids, dipeptides with terminal amino groups Tyr, Arg, Glu, Met. Low-molecular-weight peptides were presented by met-enkephalin, arginine-vasopressin, angiotensin group, oxytocin, leu-enkephalin, and gastrin fragments. Met-enkephalin was most characteristic of the brain and less so of the kidneys and liver. Peptides belonging to the neurokinin group predominated in the brain; oxytocin and arginine-vasopressin were also identified. These peptides in trace amounts were detected in the lungs, liver, and heart. Compounds of the neuromedine family (B, C, N, K) and bombesins were identified in all tissues.

Hence, human fetal tissues and rabbit juvenile tissues can be regarded as rich construction material with high biochemical potential.

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