BIOGERONTOLOGY

Fluorescent Microscopic Study of Epithalon Binding in Maternal and Fetal Rabbit Tissues in Health and under Conditions of Placental Insufficiency

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 5, pp. 578-581, May, 2005
Original article submitted May 18, 2004

Epithalon (regulatory tetrapeptide) labeled with dansil (fluorescent stain) easily penetrates into all tissues and organs of pregnant rabbit females and through the placenta into fetal organs. Incorporation of labeled epithalon in placental tissues is more often observed in fetuses developing under conditions of placental insufficiency than in normal fetuses.

Key Words: *epithalon; regulatory peptides; placenta; placental insufficiency; placental barrier*

Regulatory peptides play the key role in regulation and realization of body functions and attract great attention of scientists because of the problem of correction or prevention of consequences of pre- and postnatal shock states, including placental insufficiency (PI) [7].

The thymus and epiphysis were the first organs from which fractions of low-molecular-weight regulatory peptides with high biological activity were isolated. One of these peptides was epithalamine, an extract from the epithalamus-pineal gland region of the brain, obtained by acetic acid extraction. The preparation contains a complex of polypeptides, but no high-molecular-weight substances [4]. It is tropic to the epithalamus-pineal gland region of the brain, modulates activity of the hypothalamic-pituitary and of other organs [3].

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Epithalon is a tetrapeptide (Ala-Glu-Asp-Gly) created on the basis of amino acid structure of epithalamine at St. Petersburg Institute of Bioregulation and Gerontology. Epithalon can possess higher biological activity than its precursor [9]. Geroprotective, antioxidant, and antitumor effects of epithalon are known [8,9]. Presumably, epithalon is one of bioactive factors regulating the epiphysis work. Peptide fragment Ala-Glu-Asp-Gly can be found in endogenous regulatory proteins and peptides (prothymosine, parathymosine, cytostatine, troponine, thyroglobulin, neuron adhesion molecule, GAP-43 and P-57 calmodulin-binding proteins). Hence, it is quite possible that epithalon forms in the cells as a result of proteolysis of the precursor protein.

The data on epithalon effects describe mainly its oncostatic and antioxidant properties. The mechanisms underlying the effect of the peptide on cells and regulatory systems remain unknown, but some reports contain indirect evidence of the existence of a receptor for this tetrapeptide, for example, extremely low concentration of the agent needed for manifestation of its

0007-4888/05/1395-0615

regulatory effects [6,8]. Study of epithalon binding in maternal and fetal tissues and its penetration through the placental barrier seems to be important.

MATERIALS AND METHODS

Experiments were carried out on 12 adult female Chinchilla rabbits (4.5-5.0 kg) kept under standard vivarium conditions at natural illumination on balanced ration. For paired comparison of the reactions of fetuses developing under conditions of normal blood supply and impaired uteroplacental bloodflow, on day 18 of pregnancy a surgical intervention was carried out under ether mask narcosis. About one-third of placental vessels were ligated in one uterine horn for every fetal sac, thus creating dosed reduction of the bloodflow to the maternal part of the placenta, this leading later to delay of fetal development in this uterine horn. The second uterine horn remained intact. At the end of pregnancy (on day 29) experimental animals were intravenously injected with epithalon labeled with a fluorescent dye (30 μg/kg). Epithalon covalently bound at N-terminal to dansil (fluorescent stain, maximum $\lambda_{absorb.}$=330 nm, $\lambda_{emis.}$=530 nm). The molecular weight of the preparation is 622,34 amu), purity according to high-pressure liquid chromatography) at least 95%.

One hour after injection the animals were sacrificed by air embolism. Morphological specimens of female organs, normal fetuses, and fetuses developing under conditions of placental insufficiency were collected. Fragments of maternal organs (liver, lung, brain, stomach, intestine) and fetal organs (liver, lung, brain, stomach, intestine, and placenta) were frozen and used for making cryostat sections. The sections were analyzed on the same day under a Zeiss Axiostar plus fluorescent microscope.

RESULTS

Dansil-labeled epithalon circulated free in maternal body. Epithalon binding was detected in all studied maternal tissues. The fluorescence was mainly even and bright or focal with predominant localization of the label on cell membranes (Fig. 1).

Dansil-bound epithalon penetrated into all studied tissues of normal fetuses (Fig. 2) and fetuses developing under conditions of placental insufficiency (Fig. 3). The fluorescence in the lungs, liver, gastrointestinal tract was brighter and more intensive than in placental and cerebral tissues, where the fluorescence was weak and diffuse. Incorporation of labeled epithalon in placental tissue was more often observed in placental insufficiency than in normal fetal development.

No characteristic fluorescence of the label was seen in maternal tissues and control fetal tissues.

Our results indicate free penetration of epithalon into virtually all maternal and fetal tissues, which can be explained by its small size and low molecular weight.

The effects of regulatory peptides on the pre- and postnatal status of mammalian progeny are how actively studied. A single preventive intraperitoneal injection of semax in doses of 0.05 and 0.10 mg/kg significantly reduced hypoxia-induced bradycardia in 14- and 21-day-old rats and had an antiarrhythmic effect, removed the effects of hypoxia on the parameters of the orientation and exploratory behavior during the posthypoxic period [7]. Injection of mepyridine (opioid receptor agonist) in a dose of 2 mg/kg into the

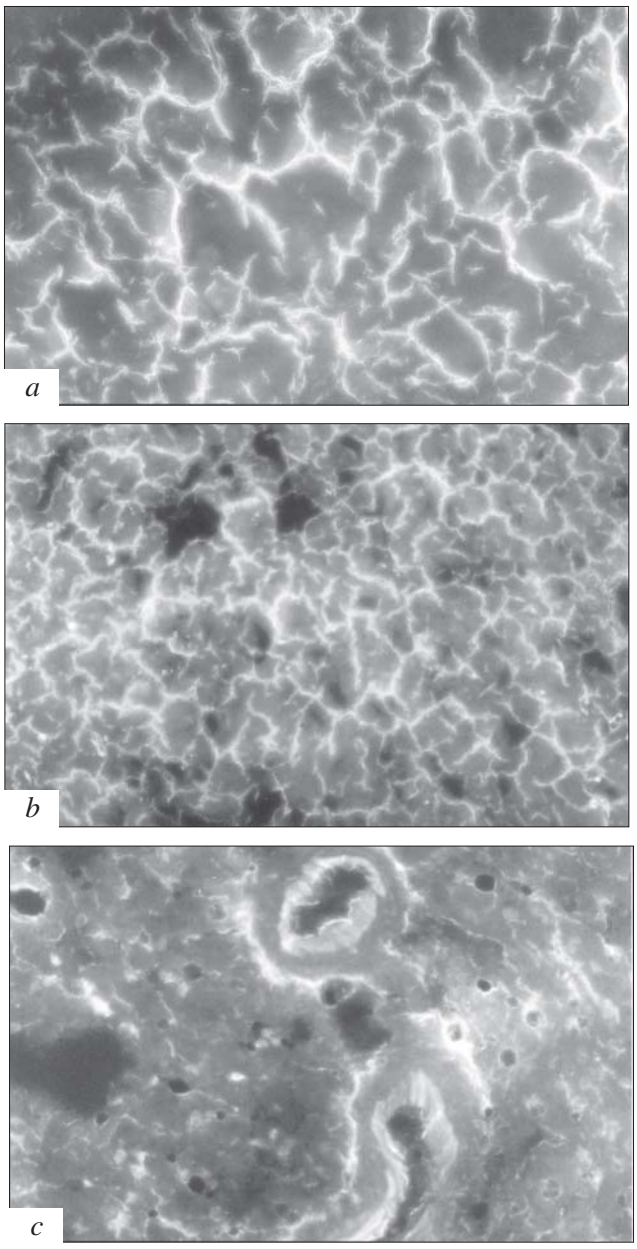

Fig. 1. Distribution of fluorescent signal in lung (*a*), intestinal (*b*), and liver (*c*) tissues of rabbit females. ×330 (*a*, *c*), ×220 (*b*).

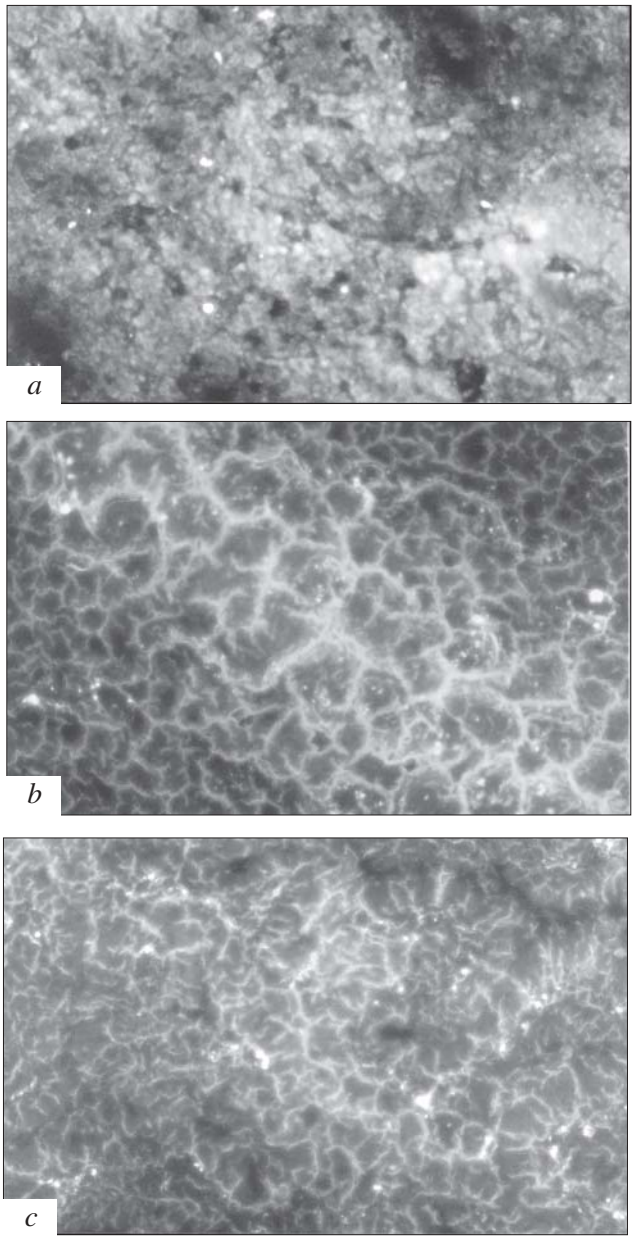

Fig. 2. Distribution of fluorescent signal in cerebral (*a*), gastric (*b*), and placental (*c*) tissues of a normal fetus. ×110 (*a*, *c*), ×220 (*b*).

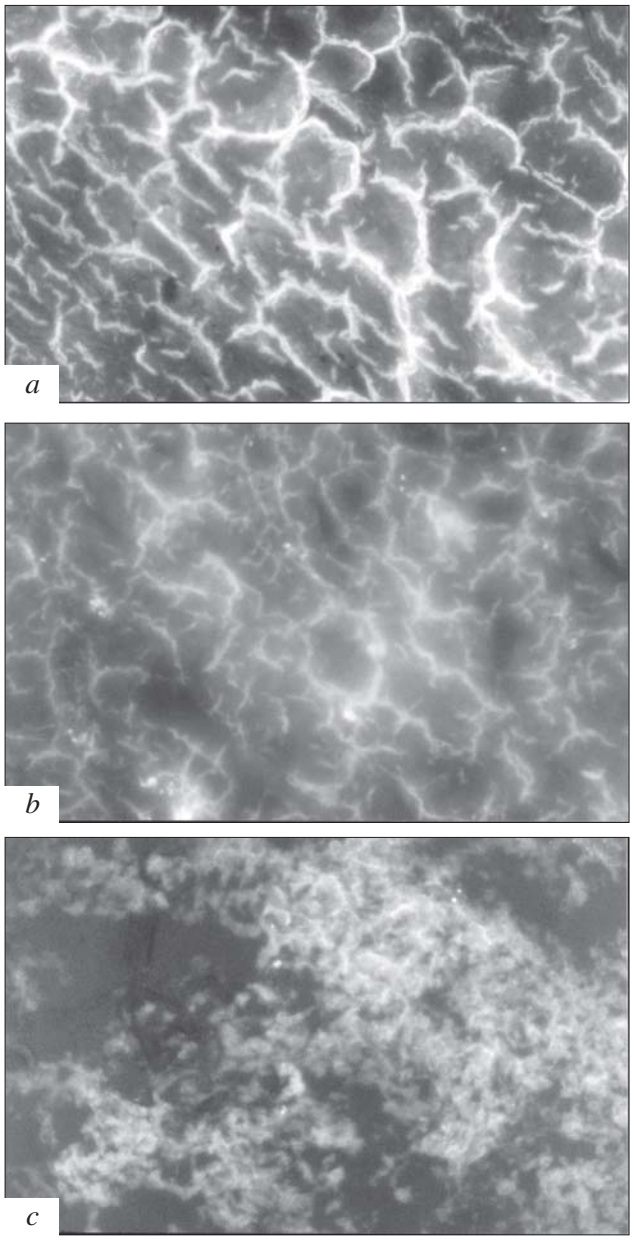

Fig. 3. Distribution of fluorescent signal in lung (*a*), intestinal (*b*), and placental (*c*) tissues of a fetus with placental insufficiency. ×330 (*a*, *b*), ×110 (*c*).

uterus of monkeys led to disappearance of the majority of negative effects in the progeny [10]. Thyrotropin-releasing factor injected in a single dose of 50 mg to preterm children with hypoxic damage to the brain increased blood pressure and normalized cerebral circulation in the major arteries.

Up to 65% children with prenatal history of placental insufficiency suffer from some disease, dysadaptation, or delayed development; the incidence of CNS injuries reaches 30% in this population. Placental insufficiency leads to impairment of the transport, trophic, endocrine, metabolic, and other important functions of the placenta. Placental insufficiency of any origin is caused by impairment of placental circulation, including microcirculation, and metabolic processes, which are closely related to each other and are mutually dependent [5]. The regulatory peptides characterized by extremely high physiological activity can act as mediators and, appearing in the bloodflow, can function as effector physiological agents or modulators of metabolism, *i.e.* as true hormones [1,2]

Our experiments demonstrated rapid incorporation of epithalon in metabolic processes in virtually all maternal and fetal tissues in normal pregnancy and in preg-

nancy aggravated by placental insufficiency, which prompts further investigation of the regulatory effects of this peptide in the mother—placenta—fetus functional system.

The study was supported by the Ministry of Education of Russia (grant No. PD02-1,4-159), Foundation for Promotion of National Science, Russian Foundation for Basic Research (grant No. 02-04-49873).

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