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ORIGINAL ARTICLE

Xeneostrogenes influence on cholinergic regulation in female rats of different age

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In view of the threat of adolescent children diet contamination, as a particularly sensitive population to chemicals with endocrine properties, an analysis of the occurrence of their health impairment risks in the future is necessary. The aim of the presented work was the study of the effect of exogenous estrogens on acetylcholinesterase activity in the organs of female rats of different ages. At the beginning of the experiment, the age of the experimental animals was 3 months-in the pubertant period and 6 months-mature ones. To modeling the effect of exogenous estrogen *in vivo*, rat's food was treated with Synestrol in the calculation of 2 μg per kg for 45 days*. In vitro*, Sinestrol was added to the test samples at a concentration of 0.5 nmol/L, followed by incubation for 1 hour. At alimentary estrogen influence AChE study showed that in pubertal rats its activity is higher by 22% compared to the control indices in the brain. The enzyme activation was observed by 15% in the mature females.In the blood serum and liver tissue, the enzyme activity inhibition is determined. The study of the effect of synthetic estrogens on the activity of AchE *in vitro* did not reveal significant changes in the indices in all the studied tissues of animals of both experimental groups. Deterministic tension in the cholinergic mediator system of the brain against the background of AChE inhibition in the blood serum and liver tissue with predominance in the pubertal females in comparison with the affects in sexually mature rats indicates a lower efficiency of mediator transmission in the corresponding cholinergic neurons of the younger animals, which justifies the existence specific age-related physiological conditions determining high sensitivity to exogenous estrogen-like compounds.
Keywords: Xenoestrogens; acetylcholinesterase; *in vivo*; *in vitro*; brain health disorders

Introduction

The effects of many contaminants are associated with their potential estrogenic effect. In nature there are some 300 natural compounds, and even more anthropogenic compounds, which have estrogenic or anti-estrogenic activity (Shore and Shemesh, 2016). These substances are classified as compounds destroying the endocrine system and including many classes of organic compounds. These agents change the hormonal and homeostatic systems and can act through different mechanisms simultaneously. Although the influence of the majority of xenoestrogens has been recognized for many years as "weak" because of their inability to cause transcription effects, now it has been proven that they are quite potent signal initiators of cascades emerging from the membrane (De Coster and van Larebeke, 2012).

This is due to the modern living conditions, namely, increased pollution of the atmosphere, malnutrition and other negative factors. Estrogens are classified by the World Health Organization as carcinogens of group I and ones are of considerable interest, because they are found in surface waters around the world and long-term exposure to estrogen-borne water can disrupt the sexual development of living organisms. Xenoestrogens are represented by industrial chemicals (for example, DDT, phthalic acid esters, bisphenol A), antimicrobial (zearalenone) and medical drugs, personal hygiene products (Rubin, 2011). Ones are determined in drinking and bottled water (Wielogórska et al., 2015), present in food products-cereals, vegetables and fruits (Massart and Saggese, 2010), livestock products-meat, milk, eggs (Wielogórska et al., 2014), as well as products containing phytoestrogens (He et al., 2014). These hormones in the environment were associated with changes in microbial ecology, increased proliferation of antibiotic resistance toward pathogens, toxicological effects in on aquatic species, and a negative effect on human health (Shore and Shemesh, 2016; Lykholat et al., 2016*a*).

Food is one of the main routes of exposure to estrogen per person from the environment. Estrogens are also used in animal husbandry to increase growth in steers. Hormones are not completely destroyed during the heat treatment of foods. Therefore, all the hormones initially presenting in meat, milk, eggs, vegetables and fruits remain there, although sometimes in smaller doses. Steroid hormones are destroyed by heat treatment least of all. Some food estrogens may occur during improper cooking (smoking, roasting). Getting into the human body with food, hormones are perceived by them as their own. In 1988, the FAO/WHO Expert Committee on Food Additives declared that the remnants of sex hormones, usually present in

animal products, do not pose a threat to human consumption. Nevertheless, the risks associated with the alimentary effects of synthetic hormones have not yet been fully characterized, and publications give conflicting reports about the long-term consequences of such products use (Omoruyi et al., 2013).

Despite the strict legislation of the European Union (EU) on the need to ensure the safety of infant food supplies, taking into account the xenoestrogens residual content concentration low levels, there is a threat of the residual substances in food for children (Hercegová et al., 2007). In experiments on animals, it has been proved that these compounds regulate various biochemical pathways, while significantly affecting steroid receptors and growth factors, remodeling of angiogenesis and tissues, steroid synthesis, proliferation, apoptosis. The regulation of some genes, depending on the age occurs in opposite directions (Lykholat et al., 2016*b*).

The onset of the period of puberty is characterized by a significant increase in the synthesis of estrogen in the ovary as a result of a change in the "adjustment" of the hypothalamus, which "allows" higher concentrations of the hormone in the blood. Increased levels of estrogen circulating in the blood, affects many body systems, resulting in various physiological changes.

Acetylcholinesterase (AChE, 3.1.1.17) is localized in the gray matter of the brain, heart, lungs, intestines, spleen, liver, interneuronal synapses, autonomic nervous system ganglia, and erythrocyte membranes. Enzyme plays a key role in the processes of neurohumoral and synaptic transmission: it catalyzes the hydrolysis of acetylcholine and thereby stops the mediator from affecting the cholinreceptor which is responsible for the permeability of the postsynaptic membrane for ions in the cholinergic synapses. The acetylcholine-acetylcholinesterase system is one of the main links in the autonomic nervous system, through which other mediator and modulator processes carrying out the corresponding reactions of the effector organs to the central nervous impulses are involved. At AChE suppressing very slow acetylcholine release from the receptors (through diffusion) occurs, which leads to a disruption in the transmission of nerve impulses and can result in serious disorders of the vital activity of the organism (Starostina and Degteva, 2008).

In particular, when some insecticides and pesticides are poisoned, AChE activity is inhibited, followed by changes at the nervous system level, expressed by increased cholinergic stimulation, alteration of molecular pathways, initiation of proliferation, and carcinogenesis of the breast (Cabello et al., 2001). Phytoestrogens increased the acetylcholinesterase activity of rat neuronal cells by binding to estrogen receptors (Isoda et al., 2002).

Consumers are exposed to estrogen-like substances low levels influence throughout life. The effect of these compounds is currently assessed with an emphasis on mutagenicity and genotoxicity. However, this approach does not take into account the integration of the latest new toxicology studies, for example, in endocrine disorders, toxicity mixtures and toxicity at development. In view of the threat of adolescent children diet contamination, as a particularly sensitive population to chemicals with endocrine properties, an analysis of the occurrence of theirhealth impairment risks in the future is necessary.

The aim of the presented work was the study of the effect of exogenous estrogens on the activity of AChE in the organs of female rats of different age.

Methods

To modeling the effect of exogenous estrogen *in vivo*, rat's food was treated with Synestrol (meso-3.4-di-(para-hydroxyphenyl)-hexane as derivative of stilbene, differing chemically from steroid estrogen hormones (female sex hormones), but according to biological and the healing properties is close to them in the calculation of 2 μg per kg for 45 days. At the beginning of the experiment, the age of the experimental animals was 3 months-in the pubertant period (group II, n=6) and 6 months-mature ones (group IV, n=6). Control groups were intact animals of the corresponding age (groups I, n=6 and III, n=6).

The animals were withdrawn from the experiment under ketamine anesthesia (1 mg per 100 g) by decapitation the morning of the next day after the experiment last procedure end. The studies were carried out in accordance with the requirements of Directive No. 2010/63/EC on the protection of animals used for scientific purposes.

In vitro, Sinestrol was added to the test samples at a concentration of 0.5 nmol/L, followed by incubation for 1 hour. A corresponding aliquot of physiological solution was added to the control samples.

The test tissues, such as brain and liver, were washed with a cooled physiological solution, ground to obtain 10% homogenates in the cold and homogenized in a fivefold volume of 0.25 M sucrose prepared in 0.001 M EDTA solution.

Cholinergic regulation was assessed by the activity of acetylcholinesterase in tissues and blood serum, which was determined spectrophotometrically by Ellman's method using as substrate-acetylthiocholine, 5,5-dithiobis(2-nitrobenzoic acid) as a thiol group indicator with registration of optical density at a wavelength of 412 nm (Ellman et al., 1961).

Statistical processing of results was carried out using the Statistica 6.0 application software package (StatSoft, USA) using parametric Student's t-test. Changes between the compared indexes was considered significantat $p < 0.05$.

Results

The activity of membrane-bound acetylcholinesterase, cell stability, anti-oxidative activity, and generation of free radicals are parameters using to characterize the structural and functional changes in the cell. Neurotoxicity is one of the most serious toxicological problems, since damage to even neurons small number can have unpredictable consequences for the whole organism. When studying the effect of synthetic estrogens entering the body with food, the following results were obtained.

In the brain AChE study showed that in pubertal rats its activity is higher by 22% compared to the control indices. The enzyme activation was observed by 15% in the mature females group (Table 1).

Table 1. Effect of alimentary xenoestrogens on acetylcholinesterase activity (*M* ± *m*, *n*=6).

The samples	I group	II group	III group	IV group
Blood serum, mM/mg protein/L	4.52 ± 0.31	4.07 ± 0.28*	4.74 ± 0.36	4.40 ± 0.33
Brain, mM/mg protein/min	13.35 ± 0.80	16.23± 0.92*	14.06± 0.95	16.17±1.03*
Liver, mM/mg protein/min	2.77 ± 0.21	2.53 ± 0.25*	2.91 ± 0.18	2.73 ± 0.22

Notes: *-changes significant compared with the control group, $p < 0.05$.

Table 2. Effect of synthetic estrogens on acetylcholinesterase activity *in vitro* (*M* ± *m*, *n*=6).

The samples	I group	II group	III group	IVgroup
Blood serum, mM/mg protein/L	4.52 ± 0.31	4.50 ± 0.36	4.74 ± 0.36	4.75 ± 0.34
Brain, mM/ mg protein /min	13.35± 0.80	13.41 ± 0.93	14.06 ± 0.95	14.04 ± 1.05
Liver, mM/ mg protein /min	2.77 ± 0.21	2.74 ± 0.17	2.91 ± 0.18	2.90 ± 0.25

Notes: *-changes significant compared with the control group, $p<0.05$.

In the blood serum and liver tissue, the enzyme activity inhibition is determined. In pubertal animalsthe indices were significantly different from those of the corresponding control (by 10% and 9%, respectively). For sexually mature females, the activity deviations was a little lesspronounced (by 9% in both investigated organs).

The study of the effect of synthetic estrogens on the activity of AchE in vitro did not reveal significant changes in the indices in all the studied tissues of animals of both experimental groups (Table 2).

Discussion

One of the most important aspects of organism integral functioning is the presence of contact molecular mechanisms of signaling systems providing the physiological and biochemical effects realization of hormones and neurotransmitters. Violation of neuroendocrine homeostasis by chemical substances can lead to a number of physiological changes. During the development of an individual from a single cell to prenatal stages to adolescence to adulthood and through the complete life span, humans are exposed to countless environmental and stochastic factors, including estrogenic endocrine disrupting chemicals. Brain cells and neural circuits are likely to be influenced by estrogenic endocrine disruptors (EEDs) because they strongly dependent on estrogens (Preciados et al., 2016).

At the level of the nervous system changes increase cholinergic stimulation and, probably, change molecular pathways. The observed changes in their physiological significance are compensatory. According to the results of the experiment and the literature data (Kozhura et al., 2007) these shifts are excessive for cerebral neural cells of female rats, resulting in a structural reorganization of nervous tissue, and can initiate the appearance of new pathological states, in particular, produce changes in the epithelium of the breast, affecting the process of carcinogenesis in the future by increasing cholinergic stimulation. Preservation of activating influences on the brain proves its stability: the enzyme activation is evidence of high functional plasticity of the brain tissue. At the same time, the tension in the cholinergic mediator system was higher in younger rats. In the developing brain this phenomenon is able to directly disturb a number of processes in the nervous system, including neuronal proliferation and differentiation, glycogenesis and apoptosis (Flaskos, 2012).

In blood serum and liver AChE activity decrease in can be an indicator of worsening of liver synthesizing function, in particular stagnant phenomena in the liver (due to hemodynamic disorders) and kidneys.

The noted inhibition of AchE as the enzyme responsible for the hydrolysis of acetylcholine in the cholinergic synapses may be one of the mechanisms of exposure to xenoestrogens. When AChE is suppressed, very slow release of acetylcholine from the receptors (by diffusion) occurs, which leads to the nerve impulses transmission disruption. This can cause serious disorders in the life of the body.

Conclusions

Deterministic tension in the cholinergic mediator system of the brain against the background of AchE inhibition in the blood serum and liver tissue with predominance in the pubertal females in comparison with the affects in sexually mature rats indicates a lower efficiency of mediator transmission in the corresponding cholinergic neurons of the younger animals, which justifies the existence specific age-related physiological conditions determining high sensitivity to exogenous estrogen-like compounds. Due to changes in the rate of detoxification reactions, and not the metabolism of estrogens entering the body, in particular with food, animals become less sensitive to the effects of these substances with age. Such phenomena can later become triggers for reducing the potential of compensatory mechanisms by regulating brain homeostasis, as well as directly affecting intracellular processes during the growing and differentiation of cells, with subsequent possible health problems, in particular, may contribute in the development of complex chronic brain health disorders in the future.

Further studies are needed to create more targeted preventative interventions as well as specific, effective therapeutics to decrease the affects of xenoestrogens on human health, and first of all, children's population.

References

Cabello, G., Valenzuela, M., Vilaxa, A., Durán, V., Rudolph, I., Hrepic, N. & Calaf G. (2001). A rat mammary tumor model induced by the organophosphorous pesticides parathion and malathion, possibly through acetylcholinesterase inhibition. Environmental Health Perspectives, 109(5), 471-479.

De Coster, S., & van Larebeke, N. (2012). Endocrine-disrupting chemicals: associated disorders and mechanisms of action. Journal of Environmental and Public Health, 2012: 713696. doi: 10.1155/2012/713696

Ellman, G. L., Courtney, K. D., Andres, V. Jr., & Feather-Stone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 7(1), 88-95.

Flaskos, J. (2012). The developmental neurotoxicity of organophosphorus insecticides: a direct role for the oxon metabolites. Toxicology Letters, 209(1), 86-93. doi: 10.1016/j.toxlet.2011.11.026

He, X., Xi, C., Tang, B., Wang, G., Chen, D., Peng, T., & Mu, Z. (2014). Simultaneous determination of 30 hormones illegally added to anti-ageing functional foods using UPLC-MS/MS coupled with SPE clean-up. Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment, 31(10), 1625-1638. doi: 10.1080/19440049.2014.953602

Hercegová, A., Dömötörová, M., & Matisová, E. (2007). Sample preparation methods in the analysis of pesticide residues in baby food with subsequent chromatographic determination. Journal of Chromatography A, 15, 1153(1-2), 54-73. doi: 10.1016/j.chroma.2007.01.008

Isoda, H., Talorete, T. P., Kimura, M., Maekawa, T., Inamori, Y., Nakajima, N., & Seki, H. (2002). Phytoestrogens genistein and daidzin enhance the acetylcholinesterase activity of the rat pheochromocytoma cell line PC12 by binding to the estrogen receptor. Cytotechnology, 40(1-3), 117-123. doi: 10.1023/A:1023903220539

Kozhura, V. L., Malygin, V. V., Makhayeva, G. F., Serebryakova, O. G., Novoderzhkina, I. S., & Kirsanova, A. K. (2007). A Late Response of the Cholinergic Neurotransmitter System in Acute Massive Blood Loss. General Reanimatology, 3(4), 31-33 (In Russian).

Lykholat, O. A., Grigoryuk, I. P., & Lykholat, T. Y. (2016*a*). Metabolic effects of alimentary estrogen indifferent age animals. Annals of Agrarian Science, 14(4), 335-339. https://doi.org/10.1016/j.aasci.2016.09.012

Lykholat, T., Lykholat, O., & Antonyuk, S. (2016*b*). Immunohistochemical and biochemical analysis of mammary gland tumours of different age patients. Cytology and Genetics, 50(1), 32-41. doi: 10.3103/S0095452716010072

Massart, F., & Saggese, G. (2010). Oestrogenic mycotoxin exposures and precocious pubertal development. International Journal of Andrology, 33(2), 369-376. doi: 10.1111/j.1365-2605.2009.01009.x

Omoruyi, I. M., Kabiersch, G., & Pohjanvirta, R. (2013). Commercial processed food may have endocrine-disrupting potential: soy-based ingredients making the difference. Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment, 30(10), 1722-1727. doi: 10.1080/19440049.2013.817025

Preciados, M., Yoo, C., Roy, D. (2016). Estrogenic endocrine disrupting chemicals influencing NRF1 regulated gene networks in the development of complex human brain diseases. International Journal of Molecular Sciences, 17(12): E2086. doi: 10.3390/ijms17122086

Rubin, B. S. (2011). Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. The Journal of Steroid Biochemistry and Molecular Biology, 127(1-2), 27-34. doi: 10.1016/j.jsbmb.2011.05.002

Shore, L. S., & Shemesh, M. (2016). Estrogen as an environmental pollutant. Bulletin of Environmental Contamination and Toxicology, 97(4), 447-448. doi: https://doi.org/10.1007/s00128-016-1873-9

Starostina, V. K., Degteva, S. A. (2008). Cholinesterase: methods of analysis and diagnostic value. Vektor-Best, Novosibirsk (In Russian).

Wielogórska, E., Elliott, C. T., Danaher, M., & Connolly, L. (2015). Endocrine disruptor activity of multiple environmental food chain contaminants. Toxicology *in vitro*, 29(1), 211-220. doi: 10.1016/j.tiv.2014.10.014

Wielogórska, E., Elliott, C. T., Danaher, M., & Connolly, L. (2014). Validation and application of a reporter gene assay for the determination of estrogenic endocrine disruptor activity in milk. Food and Chemical Toxicology, 69, 260-266. doi: 10.1016/j.fct.2014.04.028

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