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Serum Amino Acid Levels in Rats under Long-term Administration of Progesterone and Melanin Treatment

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Introduction. Obesity is one of the most common complex health problems in the world. Main reasons of the development of obesity are marketing unhealthy food and switching from active to sedentary lifestyles [1]. Each year at least 2.8 million people die as a result of being overweight or obese. According to statistics, 44 % of diabetes, 23 % of ischemic heart disease and 7-41 % of certain cancers are consequences of overweight and obesity [2].

Antiobesity strategies need to overcome the strong homeostatic mechanisms facilitating the reduced energy expenditure. Accordingly, much effort has been devoted in this direction during the last two decades, with the recognition of the major metabolic roles of numerous neuropeptides and transmitters as well as multiple peripheral hormones [3-5], not only in the regulation of feeding but also in the physical activity.

The use of drugs based on steroid hormones, progesterone in particular, can be one of many factors contributing to obesity. It is known that progesterone which enters the female body artificially, leads to fat accumulation effect [6]. Even in normally cycling women, the elevated body mass index (BMI) is associated with shorter luteal phase and lower progesterone levels. The underlying mechanisms by which obesity impairs progesterone production are not fully elucidated. The hypothetical mechanism may be the following: the alteration in luteinizing hormone levels induced by obesity diminishes the progesterone production by the corpus luteum [7, 8]. A lack of the accurate and scientifically recognized explanation of the mechanism of the fat increase causes a difficult situation which is currently present in the treatment of obesity.

Some of the most thoroughly studied natural genesis of macromolecular structures except for proteins and nucleoproteins are carbohydrate and phenolic biopolymers. Melanin occupies a special place among natural polymers. Melanin is chemically relatively inert, but at the same time it is the only polymer with biological properties of a stable free radical. Characteristic features are the presence of highly melanin paramagnetic centers, a variety of functional groups as well as systems of conjugated bonds in their molecules. These characteristics determine the structure of their photo- and radioprotective, antioxidant and other properties that is why melanin is widely used in medicine, pharmacology, cosmetology, agriculture and food industry [9].

In a number of studies, metabolites such as branched chain amino acids (BCAAs) and aromatic amino acids (AAAs) have been consistently associated with risks of obesity, insulin resistance and type 2 diabetes [10]. In this article

the main attention is paid to the molecular and clinical associations between melanin and serum amino acids under progesterone-induced obesity. Revealing of these interactions and mechanisms may improve our understanding of the complex treatment of obesity.

Materials and methods. The research was conducted in compliance with the standards of the Convention on Bioethics of the Council of Europe "European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes" (1986). The general ethical principles on animal experiments, approved by the First National Congress on Bioethics in Ukraine (September 2001) and other international agreements and national legislation in this field. Animals were kept in a vivarium that was accredited in accordance with the "sanitary rules for arrangement, equipment and maintenance of experimental biological clinics (vivarium)". Instruments used for the research were subjected to the metrological control.

In the present study white nonlinear female rats weighing 210 ± 20 g were used at the beginning of the experiment. The animals of each experimental group were kept in polypropylene cages in an environmentally controlled clean aired room with the temperature of $22 \pm 3^\circ\text{C}$, a 12 h light/12 h dark cycle and a relative humidity of $60 \pm 5\%$. The studies were conducted on 30 rats that were divided into three groups of 10 animals each: 1 – a control group; 2 – a progesterone-induced obese group; 3 – a progesterone-induced obese group under melanin treatment.

The progesterone oil solution (Biopharma, Kyiv, Ukraine) was administered in the dorsal neck region of rats (10 mg/kg body weight, treated daily during 28 days) to modulate obesity. Rats belonged to the control group were injected with the oil used for the progesterone administration.

The progesterone solution (10 mg/kg body weight, treated daily during 28 days) was administered to the third group of rats. Then this group received aqueous solution of melanin in dose 1 mg/kg at volume 2 ml/kg per os (p.o.). Melanin was obtained from yeast-like fungi *Nadsoniella nigra* X1 strain from the Ukrainian Antarctic station.

To confirm the development of obesity we have determined the body mass index and Li index. Blood serum was taken after the 28 days of progesterone injection to determine amino acid levels.

Amino acid blood serum levels were determined by using ion exchange chromatography analyzer (Spekman, Stein, Moore).

The statistical analysis of data was carried out by the software package "Statistica 7.0". For the analysis of data distribution type, Shapiro-Wilks' criterion was used. We used Student's t-test for independent samples. Mean values (M) and standard deviations (SD) were calculated. A significant difference was considered at $p \leq 0.05$.

Results and discussion. We determined amino acid levels of the rats under progesterone long-term administration and melanin treatment. BSAAAs contain about 40% of essential amino acids and 35 % of muscle amino acids. The role of BCAA in hormonal regulation processes is very important because this amino acid increases the secretion of insulin by pancreatic β -cells and activates mTOR signal transduction pathway. Accordingly, the measurement of serum BCAA levels was the first stage of our investigation.

It has been established that valine, leucine levels in the progesterone group were 1.58, 1.5 times lower than in the intact group and the isoleucine level has not been changed significantly. The isoleucine level has decreased after melanin treatment (Fig. 1).

The data show the mean $\pm$ SD of 10 animals; asterisk (*), $p < 0.05$ in comparison with the intact group; asterisk (#), $p < 0.05$ in comparison with the progesterone group

BCAAs are essential amino acids that cannot be synthesized de novo in organisms. The level of circulating BCAAs could be caused by a dietary intake

and by the degradation of protein in tissues. Previous studies showed that increasing the level of BCAAs leads to positive effects that improve metabolic parameters such as body composition, glycaemia levels and satiety [11]. Also, BCAAs control hormone is released in the gastrointestinal tract and in fat deposits. Treatment with leucine for six weeks has increased adiponectin and decreased cholesterol in plasma of the previously obese mice, without changing body weight or fat mass [12]. On the other hand, previous studies showed the association between insulin resistance, development of type 2 diabetes and increased circulating BCAAs levels [13].

Fig. 1. Valine, isoleucine, leucine levels in serum of rats under progesterone long-term administration and melanin treatment in comparison with the intact group

The data show the mean $\pm$ SD of 10 animals; asterisk (*), $p < 0.05$ in comparison with the intact group; asterisk (#), $p < 0.05$ in comparison with the progesterone group.

The research has shown that lysine, histidine, methionine blood serum levels have changed significantly after melanin treatment in comparison with the progesterone group of rats (Fig. 2).

Fig. 2. Lysine, histidine, arginine, threonine, methionine levels in serum of rats under progesterone long-term administration and melanin treatment in comparison with the intact group

The decrease of essential amino acid levels in blood serum of animals under the long-term administration of progesterone is in line with the previous studies [14]. The data about the increase in the concentration of l-homoarginine in the maternal plasma during human pregnancy have been reported [15]. This observation along with a well-known function of homoarginine, the methylene homologue of l-arginine as a substrate for nitric oxide (NO) synthase, was the ignition for the start of intense research on the physiology and pathology of homoarginine. Also, the circulating concentration of homoarginine was found out to be lower in patients suffering from various diseases and homoarginine emerged within only very few years as a novel cardiovascular risk factor [15]. The concentration of progesterone also increases during pregnancy. This natural correlation can explain the effects of progesterone long-term administration on homoarginine and arginine levels.

The next stage of our research was to investigate aromatic amino acid concentrations in blood serum of rats under artificial progesterone-induced obesity (Fig. 3).

Fig. 3. Glutamic acid, glycine, tyrosine, phenylalanine levels in serum of rats under progesterone long-term administration and melanin treatment in comparison with the intact group

The data show the mean $\pm$ SD of 10 animals; asterisk (*), $p < 0.05$ in comparison with the intact group; asterisk (#), $p < 0.05$ in comparison with the progesterone group.

Melanin treatment has led to the increase of glutamic acid, glycine and tyrosine blood serum levels in obese group of rats.

Aromatic amino acids are the substrates for synthesis of neurotransmitters. Changes in the content of aromatic amino acids can be a reason of disfunction of synthesis of these neurotransmitters. The significant decrease of some amino acids, BCAAs and aromatic amino acids in particular, can show disorders of transport processes and metabolism of amino acids. This, directly or indirectly, leads to disorders in a row of neurotransmitters and hormonal systems. Also, the increased circulating levels of tyrosine and phenylalanine have been reported to be associated with insulin resistance, type 2 diabetes or cardiovascular diseases. The positive correlation between tyrosine, phenylalanine and insulin secretion may be involved in pathways to compensate the early stage of insulin resistance through stimulating insulin secretion [16].

Conclusion. Melanin may play an important role in adipose tissues of obese people as a scavenger of free radicals excessively produced in the tissues. This statement can be partially confirmed by the results of the study which have

shown the α -melanocyte-stimulating hormone (α -MSH) which is known to be a stimulator of melanogenesis that induces the weight loss in healthy people [17].

The influence of melanin on the amount of amino acids in blood serum of obese rats could suggest its role in mechanisms of obesity. The clear understanding of this role may lead to the further use of the melanin-containing solution in anti-obesity treatment.

Prospects for further research include the analysis of tryptophan and serotonin levels in rat brains under progesterone long-term administration and melanin treatment, the influence of tryptophan and serotonin levels on food and obesity.

Рекомендовано до друку комісією з біоетики

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RESEARCH ARTICLE

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Background. Obesity is one of the most common complex health problems in the world. Main reasons of the development of obesity are marketing the unhealthy food, passive lifestyle, hormonal disorders. Obesity can lead to the development of diabetes mellitus, ischemic heart disease and certain cancers. Numerous neuropeptides and transmitters as well as multiple peripheral hormones take part not only in the regulation of feeding but also in the physical activity. Peptides - serotonin, insulin, glucagon, hormones of hypothalamus and hypophysis, thyroid hormones are the examples of weight regulated hormones. The use of drugs based on steroid hormones, particularly progesterone, can also be one of many factors contributing to obesity. It is known that progesterone which enters the female body artificially leads to fat accumulation.

Phenolic biopolymers are the most thoroughly studied natural genesis of macromolecular structures. Melanin occupies a special place among natural polymers. Melanin is chemically relatively inert, but at the same time it is the only polymer with biological properties of a stable free radical. These characteristics determine the structure of their photo- and radioprotective, antioxidant and other properties that is why melanin is widely used in medicine, pharmacology, cosmetology, agriculture and food industry.

This article is focused on the molecular and clinical associations between melanin and serum amino acids under progesterone-induced obesity. Serum amino acids are a source for synthesis of hormones that have peptide and protein nature. Revealing these interactions and mechanisms of progesterone-induced obesity may improve our understanding of the complex treatment of obesity.

Materials and methods. In the current study we used white nonlinear female rats that were divided into three groups. Rats of group 1 – control; group 2 – progesterone-induced obese; group 3 – progesterone-induced obese group under melanin treatment (10 mg/kg body weight, treated daily during 28 days). Amino acid blood serum levels were determined by ion exchange chromatography analyzer (Spekman, Stein, Moore). The statistical analysis of data was carried out by the software package "Statistica 7.0". Values are statistically significant at $p < 0.05$.

Results. The recent study has shown changes in amino acid levels in blood serum of rats under progesterone long-term administration and melanin treatment. Levels of the majority of amino acid content have decreased in rats under progesterone long-term administration and melanin treatment as compared with the control group. The obtained data have given some evidence that progesterone long-term administration has a significant influence on amino acids metabolism.

Conclusion. Aromatic amino acids are the substrates for the synthesis of neurotransmitters. Changes of content of aromatic amino acids can be a reason of disfunction of the synthesis of these neurotransmitters. The significant decrease of some amino acids, particularly BCAAs and aromatic amino acids, can show disorders of transport processes and metabolism of amino acids. This, directly or indirectly, leads to weight disorders.

Key words: amino acids, melanin, progesterone, obesity.