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ROLE OF FACTORS OF SYSTEM OF OXIDE OF NITROGEN IN DYNAMICS OF FORMATION AT EXPERIMENTAL ANIMALS OF THE METABOLIC SYNDROME

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Annotation: Research objective - to investigate a nature of activity of ferments eNOS and iNOS, expressions NO and ONO2- in serum of blood of rabbits in dynamics of formation of a metabolic syndrome.

Stuff and research methods. Experiments are made on 40 rabbits-males, mass of a body from 2 - 3,5 kg. For building of model of a metabolic syndrome in a drinking bowl of animals added 5 % solution of Saccharose, and in a forage daily mixed crystal cholesterol in a dose of mass of a body of 250 mg/kg. An animal hypodermically introduced insulin in a dose 0,1 units/100 g, every other day. Duration of experiment 2 months. In experiment at development of a metabolic syndrome in blood the disbalance in NO-system, associating by an expression in serum of blood NO, iNOS and ONO2-, against activity oppression of endothelial NOS (eNOS) is positioned. Completion of supplies of L-arginina prevents the further formation at experimental animals MS, reduces to level of control of the maintenance in serum of blood of glucose, ChScom, S-peptide, and also dysfunction indexes of endothelium - level NO, eNOS, iNOS and ONO2-.

Key words: a metabolic syndrome, nitroergic system, dysfunction of endothelium.

Nitric oxide system in metabolic syndrome

The molecular and cellular mechanisms underlying endothelial damage in the development of metabolic syndrome (MS) have not been fully disclosed [1,2]. A special place in this problem is occupied by the question of the role of the relationship of nitric oxide (NO) with the components involved in its synthesis and decay [3]. Important regulators of the concentration of NO in the vascular endothelium and blood flow are the components of the NO system (NOS) - endothelial NOS (eNOS) and inducible (iNOS) [4], as well as the concentration gradient of its own NO and its relationship with other compounds, in particular, with anion superoxide (O2–) [5,6]. It is possible that the content of these components largely depends not only on the level of NO in the blood, but also on the sensitivity of the receptor structures of the vascular endothelium, the adequacy of their reactions to the action of pathogenic factors of various nature present in the systemic and interstitial blood flow. It can also be assumed that the metabolic effect of NO, its negative impact on the processes of protein, carbohydrate and fat metabolism depends on the level of individual

components of the NO system. However, the activity of NO-system components in the development of MS has not been sufficiently studied. Therefore, we decided to investigate the nature of the activity of eNOS and iNOS enzymes, the expression of NO and ONO2- in the blood serum of rabbits during the formation of metabolic syndrome.

Material and methods of research. Experiments were carried out on 40 male rabbits, weighing 2 - 3.5 kg (average weight 2.55 ± 0.814) kg. Metabolic syndrome was caused by daily adding a 5% sucrose solution to the animals' drinking bowl, and crystalline cholesterol in a dose of 250 mg/kg of body weight to the feed. Animals were subcutaneously injected with insulin at a dose of 0.1 units / 100 g, every other day. The duration of the experiment is 2 months. 3 groups of animals were identified. Each of them was injected daily intraperitoneally with an aqueous solution 10 days before the planned end of the experiment: 1st - L-arginine 50 mg/kg (Tivortin Yuriya Pharm, Ukraine), 2nd - nonselective inhibitor e NOS L-NAME (Mw-nitro-L-Arginin Methyl Ester; Sigma, USA) 5 mg/kg, 3-d –selective inhibitor of inducible NOS (i NOS) – S-MT (S-Methylisothiourea; Sigma, USA) 1 mg/kg. The appointment of modulators of L-arginine NO-synthase oxidation - L-arginine, eNOS, iNOS allows us to determine the contribution of individual components of the NO system to changes in the level of NO and its compounds ONO2 - with the development of MS [7, 8]. The control was data obtained from intact (control 1) and placebo-treated animals (instead of insulin, cholesterol and 5% sucrose, sterile twice distilled water was subcutaneously injected into the animals, and tap water was added to the drinkers (control 2).

In the blood serum (1.5 ml from the marginal vein of the rabbit ear), the content of NO was studied by its main stable metabolites – NO2- and NO3- - by the method of P.P.Golikov et al. [10], the activity of eNOS by V.V.Sumbayeva, I.M.Yasinskaya [8], the activity of iNOS and the concentration of peroxynitrite (ONO2-) – by M.Y.Ravaeva, E.N.Chuyan [11].

The total cholesterol (HCl) content was determined using a set of chemical reagents from Berlinger Mannheim (Germany) on a spectrophotometer SF-46 (Russia) at λ-500 nm. Insulin resistance was assessed by the content of C-peptide, which is synthesized by beta cells of the pancreas and secreted into the blood in an amount equivalent to insulin. The glucose and C-peptide content was determined on a Daytona biochemical analyzer from Randox (Great Britain) using special kits and a program. The study was carried out 20, 40 and 60 days after the start of the experiment.

The experiments were carried out in accordance with international standards adopted when working with experimental animals. The results of the study were processed using the application programs Statistica 6, Biostat. The data are presented in the form of arithmetic averages (M) and standard deviations (m). The Student's t-test or Wilkinson's paired test were used to compare the samples. The significance level was considered reliable at $P<0.05$. The relationship of several variables was revealed using correlation analysis by calculating the Spearman correlation coefficient.

The results of the study and their discussion. The study showed that in animals, with an increase in the duration of the pathophysiological model of MS, the content of glucose, hCG and C-peptide in the blood serum increases linearly (Table) - important indicators characterizing the degree of violation of carbohydrate and fat metabolism, a decrease in tissue sensitivity to insulin – insulin resistance (IR). A decrease in IR in animals with MS is indicated by a high level of the insulin analogue C-peptide – by 1.2; 1.3 and 1.2 times, respectively, after 20, 40 and 60 days, and glucose – by 2; 2.2 and 3.5 times. It turned out that a dynamic increase in serum NO is associated with inhibition of the eNOS enzyme, induction of the iNOS enzyme reaction rate and expression of the ONO2- concentration. It can be assumed that the

expression of NO, which increases with an increase in the duration of the experiment, is due to the induction of iNOS and an increase in the concentration of ONO2-, since the activity of eNOS was significantly reduced. It should be noted. that overexpression of NO due to iNOS induction may be associated with an increase in the content of ONO2-. Its level increases under conditions of hypoxia and the formation of the superoxide anion O2-, which oxidizes NO [4, 6]. The development of hypoxia in our experiments is evidenced by an increase in the content of hCG. with a lack of molecular oxygen in the blood: 1.7-2.5 times.

Consequently, in the development of MS in experimental animals, apparently, an important factor is the initiation of NO, due to the induction of iNOS, the expression of ONO2- and the inhibition of eNOS activity.

We administered the L-arginine modulator and inhibitors to animals to determine the role of each component of the NO–system in the development of MS. The results of the study showed that after the introduction of L-arginine (group 1), the glucose, hCG and C-peptide levels were practically normalized. At the same time, the NO level, eNOS and iNOS activity, and ONO2 content in the blood serum of animals with MS reached control values. By the end of the experiment, i.e. already with pronounced developing MS, they were within control. On this basis, it can be concluded that, apparently, an important factor in the development of MS in animals is the lack of L-arginine, since along with the normalization of the NO-system indicators, there was an improvement in the parameters characterizing the state of MS. It is possible that the replenishment of L-arginine reserves activates eNOS and, as a result, effectively utilizes the basal NO level, inhibits the activity of iNOS and, accordingly, ONO2-.

Our assumption is confirmed by the results obtained in groups 2 and 3. In group 2, blocking in the eNOS NO-system by the L-NAME inhibitor was characterized by an even greater, dynamic increase in the indicators characterizing the state of MS in animals – overexpression of NO, ONO2- and iNOS (by the end of the experiment – by 10, 17 and 8 times) against the background of 8-time inhibition of eNOS. The nonselective inhibitor also had no effect on the glucose, hCG, and C-peptide content.

With the introduction of S-MT – selective iNOS inhibitor, all the studied indicators clearly improved, but not to the level of control, i.e. they did not normalize. In order to find out due to which factors in the blood serum of animals increases the level of glucose, HCl. and C-peptide, after the end of the experiment (after 2 months), we determined the correlation between these indicators and the parameters of the NO-system. It was found that during this period, the indicators characterizing the state of MS, as well as the activity of the NO-system, were maximally disrupted compared to the control. High values of NO, iNOS and ONO2 directly correlated with high glucose, hCG and C-peptide ($r=0.84-0.88$, $P<0.001$), and reduced eNOS activity, on the contrary, had a strong inverse correlation with glucose, hCG and C-peptide ($r = -0.83$; 0.81 and 0.87, $P<0.001$).

The correlation between the state of MS and the intensity of the NO-system increased even more in animals that were injected with the drug L-NAME $r = 0.88-0.96$ and $r = 0.95 - 0.96$ ($P <0.001$), respectively, according to the terms of the study. The administration of L-arginine and S-MT to animals with MS revealed an inverse relationship between NO, iNOS and ONO2- ($r = -0.77-0.76$, $P<0.001$) and direct - eNOS ($r = 0.80-0.79$, $P<0.001$) and glucose, hCG and C-peptide indicators.

Thus, we have identified an important cause of MS development – a lack of L-arginine in the animal body. The activity of eNOS, iNOS, the content of NO, ONO2- after the preparation of S-MT not only

normalized, but also continued to deteriorate. The greatest violation of these indicators was detected when blocking eNOS - L-NAME. The analog of L-arginine steadily restored all the studied parameters to normal throughout the study. On the importance of L-arginine in the mechanisms of regulation of glucose metabolism, hCG. and the activity of the C-peptide indicates a change in the polarity of the correlation between the indicators of the NO-system and the MS development system – instead of the expected positive relationship that was observed in animals with MS and when the selective blocker eNOS - L-NAME was prescribed, the reverse value. It can be assumed that the decrease in eNOS activity is caused by two factors – a decrease in L-arginine in the animal body and a high expression of iNOS. On the other hand, the decrease in L-arginine leads to the suppression of eNOS. An adequate reaction of the endothelium, apparently, is the launch of a backup mechanism, including the activation of iNOS, as a result of which ultra-high concentrations of NO and ONO2- accumulate. According to recent studies [5, 11], the latter are associated with the development of inflammatory reactions in the vascular endothelium, increased sensitivity to biologically active compounds, damage to organs and systems responsible for hemostasis in the body.

Thus, the conducted studies have shown that L-arginine deficiency is an important factor in the dynamics of MS formation, which causes the inhibition of eNOS activity, the expression of NO, iNOS and ONO2 - which are associated with an increase in blood glucose, hCG and activation of the IR process.

Conclusions

1. The formation of metabolic syndrome in experimental animals causes an imbalance in the NO system: an increase in the serum content of NO, iNOS and ONO2 - against the background of inhibition of the activity of endothelial NOS (eNOS).
2. The correlation dependence of indicators characterizing the development of metabolic syndrome (hyperglycemia, insulin resistance, hypercholesterolemia) was revealed: strong direct ($r>0.8$) – from indicators of endothelial dysfunction (NO, iNOS and ONO2-) and strong reverse ($r>-0.8$) – from reduced eNOS activity.
3. L-arginine deficiency occupies an important place in the mechanisms of violation of the NO-system.
4. Replenishment of L-arginine reserves stops the further formation of metabolic syndrome in experimental animals, normalizes the blood serum glucose, hCG, C-peptide, as well as the initially impaired endothelial parameters – the level of NO, eNOS, NOS and ONO2- .

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