Regulatory role of endothelin-1, brain natriuretic peptide and irisin in the development of the immune response to short-term general cooling

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Abstract. The paper presents data on the study of the regulatory role of short peptides of endothelin-1, brain natriuretic peptide (Nt-pro-BNP) and irisin in the development of the immune response in humans for short-term cooling. Surveyed 73 people living in the city of Arkhangelsk, 60 of them women and 13 men aged 21 to 50 years. It has been established that the content of Nt-pro-BNP, endothelin-1 and irisin is in a wide range of fluctuations in persons living in the city of Arkhangelsk. As a result of short-term general cooling at -25 ° C for 5 minutes, three groups of individuals with a change in the content of short peptides can be distinguished: a decrease in the concentration recorded in 24.66% of the examined individuals in reactions with endothelin-1, Nt-pro-BNP and 28.77% with iris, an increase of 21.92-35.61% of individuals and no change in the content — 39.73-49.32% of those examined. Regardless of the concentration of short peptides, 20.54% of the respondents react by reducing the total content of lymphocytes and their activated forms. Improving the efficiency of catecholamine use. Low levels of endothelin-1 and irisin are associated with a high content of natural immunosuppressant IL-10. The decrease in lymphocyte content in response to general cooling occurs against the background of an increase in the pro-inflammatory cytokine TNF-α, the reaction on the part of which is accompanied by low levels of Nt-pro-BNP.

1. Introduction
A particular problem in the field of knowledge about the effect of general cooling on the human body is systemic reactions with impaired microcirculation, recycling and migration of blood cells, leading to sudden cardiovascular catastrophes that are not extremely rare. The most early reactions are from the side of catecholamines and short peptides, which alter the hemodynamics, frequency and strength of heart contractions. Short peptides, discovered in the 80s, are effective in very small doses, do not have a variety of effect, and, as a rule, initiate one action. Endothelin -1 has a powerful vasoconstrictor effect. [1-3]. Brain natriuretic peptide (Nt-pro – BNP) maintains intracellular pressure by holding sodium against a density gradient [4-6]. Irisin induces the activation of thermogenin in the cells of brown adipose tissue [7, 8]. One of the pathogenetic factors of cardiovascular crises is the systemic effect of proinflammatory cytokines. Pro-inflammatory cytokines are secreted by any cell in response to a negative factor that threatens the integrity and functional activity of the cell. The physiological effect of cytokines is local, but with an increase in their concentration in the blood, systemic damage develops. Damage to endothelial cells during an inflammatory reaction is accompanied by their retraction and the formation of gaps [9].
The change in the ratio between the content of circulating and parietal pools of blood cells is associated with changes in hemodynamics, blood viscosity and tissue perfusion [10]. Migration of cells in the tissue is provided by a significant slowdown of the blood flow velocity in the capillary network of the bloodstream [11-13]. The fastest mechanisms for regulating the state of the microcirculatory bed is the endothelial mechanism, while the endothelium-dependent vasodilation deficiency results from a shift in the balance of synthesis of nitric oxide and vasoconstrictor towards domination of vasoconstrictors, primarily endothelin-1. Nitric oxide has a cardioprotective effect by increasing coronary blood flow, reducing neutrophil accumulation, maintaining endothelium function, maintaining calcium sensitivity and contractile function without increasing energy needs and reducing myocardial oxygen consumption. However, the significance of these cardioprotective mechanisms remains completely unclear [14-17].

The objective is to establish the regulatory role of short peptides of endothelin-1, brain natriuretic peptide and irisin in the development of the human immune response to short-term general cooling.

2. Material and methods
The study involved 73 practically healthy at the time of the survey people living in the city of Arkhangelsk, of which 60 women and 13 men aged 21 to 50 years.

All studies were carried out with the consent of the volunteers and in accordance with the requirements of the Helsinki Declaration of the World Medical Association on the ethical principles of medical research (2000).

To study the effect of low-temperature factors on humans, various variants of local cold samples are widely used in physiological studies, allowing the degree of adaptive rearrangements to be assessed according to the nature of the response from different functional systems. Most often, as a cold test, immersion of the limbs in water with a temperature below 5 °C is used, as well as contact with a cold object [18, 19]. The study involves the study of systemic, rather than local reactions. Therefore, we proposed a method for examining people before and after short-term total cooling for 5 minutes at a temperature of -25 ºC. The time spent in the cold chamber was chosen empirically on the basis of a comfortable stay period. In addition, the half-life of endothelin-1 takes from 40 seconds to 4-7 minutes [20].

An immunological examination complex was carried out before and after being in the climate chamber for 5 minutes at a temperature of -25º C during the period of the minimum day length (January, February). The study of the blood concentration of a short peptide endothelin-1, cytokines IL-1β, IL-4, IL-6, IL-10, IL-17F, TNF-α was determined by enzyme immunoassay on an automatic enzyme-linked immunosorbent analyzer "Evolis" ("Bio-RAD", France), the content of lymphocyte phenotypes (CD3+, CD4+, CD8+, CD10+, CD16+, CD23+, CD25+, CD71+, CD54+, CD95+, HLA DR+) - in an indirect immunoperoxidase reaction using monoclonal antibodies ("MedBioSpectr", "Sorbent" Moscow) and flow cytometry using the Beckman Coulter Epics XL apparatus (USA) with Immunotech a Beckman Coulter Company reagents (France). The number and ratio of hemogram cells, neutrograms, monocytoograms, lymphocytograms were counted in blood smears stained by the Romanovsky-Giemsa method, the monocytoogram was determined according to O N Grigorova (1956). The selection of mononuclear cells from peripheral blood was performed according to the method of A. Boymn (1976).

Mathematical analysis of the research results was performed using the application package Microsoft Excel 2010 and Statistica 7.0 (StatSoft, USA). Check the laws of the distribution of values of immunological parameters was performed using the statistical Pearson criterion. Verification of the null hypothesis of equality of all averages in the studied groups was carried out using univariate analysis of variance. Under conditions of disobeying the data to the law of normal distribution, the comparison of two different groups by quantitative characteristics was carried out using the non-parametric Mann-Whitney test. The descriptive statistics were calculated for each of the listed indicators (M is the arithmetic average value, σ is the standard deviation, m is the standard error of the
mean, Md is the median, R is the span, W is the coefficient of variation, and the limits of the 95% confidence interval). A critical level of significance (p) was considered to be 0.05.

3. Research results and discussion

The content of short peptides of the brain natriuretic peptide Nt-pro-BNP (from 1.713 to 267.87 fmol/ml), endothelin-1 (0.073 to 7.018 fmol/ml) and irisin (0.605 to 24.328 μg/ml) are in a wide range of variations in persons living in the city of Arkhangelsk. The highest risk of reactions studied in the course of short peptides is a violation of hemodynamics as a result of extreme emissions of endothelin-1, the frequency of registration of elevated concentrations of which in persons living in Arkhangelsk is 68.5%. It is known that dysregulation of vasoconstriction is the basis of the pathology of the organs of the cardiovascular system, the most common in the North and the main cause of disability and death of Northerners. In the background of the adaptation reaction, there are problems of preserving the osmotic pressure of the cell from the natriuretic peptide, found in 11.1% of the examined individuals.

It was established that as a result of short-term cold exposure, three groups of individuals with a change in the content of short peptides can be distinguished: a decrease in the concentration recorded in 24.66% of the examined individuals in reactions with endothelin-1, Nt-pro-BNP and 28.77% with irisin, an increase of 21.92-35.61% of individuals and the absence of a change in the content — 39.73-49.32% of those examined. Regardless of the concentration of short peptides, in 20.54% of individuals, after being in a cold chamber in peripheral venous blood, there were no differences in the content of neutrophils (2.39±0.18 and 2.23±0.20 x109 cells/l), monocytes (0.38 ± 0.05 and 0.36 ± 0.06 x109 cells/l), eosinophils (0.11 ± 0.02 and 0.09 ± 0.01 x109 cells/l) in average results before and after short-term cooling however, the total leukocyte count decreases (from 4.5±0.31 to 4.1±0.35 x109 cells/l; p <0.05) due to lymphocytes (from 1.69 ± 0.12 to 1.38 ± 0.13 x109 cells/l; p <0.05), including immature T lymphocytes (from 0.40 ± 0.04 to 0.24 ± 0.03 x109 cells/l; p <0.001), T CD4+ helper (from 0.36 ± 0.04 to 0.24 ± 0.03 x109 cells/l; p <0.001), cytotoxic CD8+ (from 0.34 ± 0.03 to 0.18 ± 0.02 x109 cells/l; p <0.001), T-lymphocytes with a receptor for transferrin CD71+ (from 0.35 ± 0.03 to 0.19 ± 0.02 x109 cells/l; p <0.001), CD10 + cells capable of proliferating cells (from 0.29 ± 0.03 to 0.15 ± 0.02 x109 cells/l; p <0.001), lymphocytes with CD54 + intercellular adhesion molecules (from 0.25 ± 0.03 to 0.12 ± 0.02 x109 cells/l; p <0.001), programmed for apoptosis of CD95+ (from 0.28 ± 0.03 to 0.20 ± 0.02 x109 cells/l; p <0.01). A decrease in the content of medium lymphocytes was recorded in the structure of the lymphocytyogram (0.54 ± 0.06 to 0.40 ± 0.05 x109 cells/l; p <0.001).

The decrease in the lymphocyte count is most likely due to the redistribution of blood cells from the circulating to the marginal pool as a result of the endothelium-dependent vasodilation reaction. Inhibition of vasodilation inhibits microcirculation and provokes a disturbance of metabolic processes with the development of oxidative stress and a decrease in the bioavailability of nitric oxide [21-23]. The phase of absorption from the extracellular environment into the vessel forms a nitric oxide cycle. In the absence of secretion of nitric oxide and absorption of the endothelium cells, endothelin-1 is constantly secreted. Vasodilation failure results from the predominance of endothelin-1. In the absence of restoration of metabolism in tissues, local reactions enter into the regulation of the systemic mechanism of changes in blood pressure and the regulation of cerebral blood flow [24, 25]. The signal from the vasomotor center of the medulla oblongata increases the pressure in the proximal vascular bed to correct impaired microcirculation in the distal arterial bed. Perhaps a decrease in cells occurs due to increased lymphocyte recycling. Cells are capable of recycling due to the weak connection of lymphocytes with stromal cells of the lymphoid organs and a relatively high degree of their mobility. (especially T-cells) are actively recycled, returning to the lymphoid organs due to the “homing” mechanism, based on the affinity of the adhesion molecules of lymphocytes and endothelial cells of lymphoid organs.

In 24.66% of the examined individuals, after short-term cooling, the content of endothelin-1 decreases by 1.5–5 times in peripheral venous blood. It was established that the content of
lymphocytes and the level of their decrease depend on the initial concentration of endothelin-1: in individuals with a high initial level of peptide (1.56 ± 0.39 fmol/ml) the content of lymphocytes is significantly higher (1.80 ± 0.07 x109 cells/l) and a decrease in their concentration after exposure to cold is more pronounced (from 1.80 ± 0.07 to 1.51 ± 0.08 x109 cells/l; p <0.05), low concentrations of endothelin-1 (0.32±0.04 fmol /ml) are associated with the absence of a reaction on the part of the content of lymphocytes (1.47 ± 0.08 and 1.52±0.09 x109 cells/l). From the side of the reactions Nt-pro-BNP and irisin with the level of lymphocyte content, no such interrelationships were revealed.

Urgent systemic adaptation of a person to any adverse effect includes the reaction of catecholamines, which provide activation of the circulatory system with increased strength and heart rate, vascular tone. In the case of exposure to cold on the human body, an increase in heat generation occurs due to contractile thermogenesis and a decrease in blood circulation in the skin blood capillaries, activation of the sympathetic nervous system and its neurotransmitters norepinephrine and adrenaline [26, 27]. In our study, no increase in catecholamine content was recorded. So, after a short stay in the climate chamber of the examined individuals who react with a decrease in the concentration of short peptides, the content of noradrenaline and adrenaline does not change (181.25 ± 24.28 and 178.71 ± 23.25 ng / ml) and (77.19±6.99 and 63.01±7.08 ng/ml), respectively. In the group of individuals with an increase in the concentration of short peptides, the content of norepinephrine decreases (from 394.14 ± 32.51 to 294.42 ± 36.57 ng/ml, p<0.01). In the absence of changes in the content of endothelin-1, Nt-pro-BNP and irisin in the serum, the concentration of catecholamines also decreases: noradrenaline (from 344.63 ± 36.57 to 194.59 ± 27.03 ng/ml, p<0.01) and adrenaline (with 84.29±7.54 and 55.48 ± 6.15 ng/ml; p<0.05). It is known that catecholamines, and, in particular, norepinephrine, have caloric properties, reducing their concentration on the periphery may indicate an increase in their expenditure on increasing cell metabolism [28, 29].

Low concentrations of endothelin-1 and irisin are associated with high levels of natural immunosuppressant IL-10. In the group with a low content of endothelin-1 (0.32±0.04 fmol / ml) and irisin (2.60±0.42 µg/ml), the concentration of IL-10 (7.09±0.93 and 3.07±0.47, p<0.01 and 8.4±0.97 and 3.37 ± 0.71 pg/ml, p <0.001, respectively). It is known that IL-10 is a natural inhibitor of the immune response by reducing gene expression of differentiation molecules by T and B lymphocytes [30, 31]. Stimulation of IL-10 synthesis occurs through STAT 3 [32]. At a very high level of blood concentration of IL-10, there is a decrease in the content of cytokines IL-2. IL-4, IL-5 and TNF-α [33]. IL-10 reduces the expression of adhesion molecules on endothelial cells [34]. One of the reasons for the decrease in IL-10 secretion may be a low content of TOLLR4 on monocytes [35]. An increase in IL-10 concentrations indicates activation of inhibitory mechanisms with suppression of the expression of activating molecule genes. It is believed that the production of IL-10 requires stronger stimulation than for the secretion of other cytokines and that the threshold required for the influence of this cytokine on the proliferation of T-cells is significantly higher than that required only to preserve the cell population [35, 36]. Most likely, this inhibitory mechanism of regulation is a signal for endothelin-1 in preventing homeostasis from abrupt changes in the redistribution of cells from the circulating to the marginal pool in the vascular network under conditions of additional heat production.

In 24.66% of the examined individuals who respond to short-term overall cooling by lowering the content of brain natriuretic peptide (from 297.93±73.52 to 70.12±15.26 fmol / ml, p <0.001), there is an increase in the content of pro-inflammatory cytokine TNF- α (5.9 ±1.45 to 7.23±1.47 pg / ml, p <0.05). TNF-α plays an extremely important role in the first moments of the inflammatory reaction, since it activates the endothelium and promotes the expression of adhesive molecules, which leads to the adhesion of granulocytes to the inner surface of the vessel. Under the influence of TNF-α, the transendothelial migration of leukocytes occurs in the inflammatory focus. This cytokine activates granulocytes, monocytes, lymphocytes and induces the production of other proinflammatory cytokines - IL-1, IL-6, IFN, GM-CSF, which are TNF-α synergists. Under the influence of TNF-α, the synthesis of lipoprotein kinase is inhibited - one of the main enzymes that regulate lipogenesis. TNF-α, being a mediator of cytotoxicity, is able to inhibit cell proliferation, differentiation and functional activity of
many cells [37, 38]. It can be assumed that this level of proinflammatory cytokine in the blood is provided mainly by circulating lymphocytes.

4. Conclusion

The content of short peptides Nt-pro-BNP, endothelin-1 and irisin is in a wide range of fluctuations among people living in the city of Arkhangelsk. As a result of short-term general cooling at -25 °C for 5 minutes, three groups of individuals with a change in the content of short peptides can be distinguished: a decrease in the concentration recorded in 24.66% of the examined individuals in reactions with endothelin-1, Nt-pro-BNP and 28.77% with iris, an increase of 21.92-35.61% of individuals and no change in the content — 39.73-49.32% of those examined. Regardless of the concentration of short peptides, 20.54% of the respondents react by reducing the total content of lymphocytes and their activated forms. The content of lymphocytes and the level of their decrease depends on the initial concentration of endothelin-1: in individuals with a high initial level of peptide, the content of lymphocytes is significantly higher and the decrease in their concentration is more pronounced; low concentrations of endothelin-1 are associated with the absence of lymphocyte counts. Regardless of the reaction from short peptides to short-term total cooling, no increase in catecholamine content was recorded. Low levels of endothelin-1 and irisin are associated with a high content of natural immunosuppressant IL-10. The decrease in lymphocyte content in response to general cooling occurs against the background of an increase in the pro-inflammatory cytokine TNF-α, the reaction on the part of which is accompanied by low levels of Nt-pro-BNP.

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