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Original article

Reprint

Intracellular metabolism of peripherial blood leukocytes in arterial hypertension: experimental study

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Abstract:
Objective: to conduct a comparative analysis of the features of intracellular metabolism in polymorphonuclear leukocytes (PMN) in the peripheral blood of rats with inherited stress-induced arterial hypertension (ISIAH) vs. normotensive animals (NTA).
Materials and Methods. In the leukocytes of rat blood (15ISIAH rats and 20 NTA), the indicators of carbohydrate and lipid metabolisms, the activity of a number of key enzymes reflecting the state of redox processes, and PMN functional activity level were determined using histochemical methods.
Results. The study established that a stable increase in blood pressure was accompanied by hypertrophy of the left ventricle, degenerative changes in cardiomyocytes and a decrease in the density of the microvasculature. At the same time, the amount of intracellular glycogen in leukocytes decreased by 23% while the amount of intracellular lipids remained unchanged. The levels of activity of intracellular ATPase and myeloperoxidase decreased by 22% with a slight increase in the activity of succinate dehydrogenase (by 13%). The number of PMN with a positive hematocrit (HCT) test was higher by 35% in ISIAH rats.Conclusion. Analysis of the studied PMN metabolism indicators revealeddisorders in the energysupplying and enzymatic processes of leukocytes in arterial hypertension.

Keywords: arterial hypertension, polymorphonuclear leukocytes, intracellular metabolism.

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Introduction

For over two centuries, doctors and scientists have been concerned with the problem of arterial hypertension (AH). According to the World Health Organization, AH is currently diagnosed in 1.28 billion people 30–79 years of age [1]. Numerous clinical and scientific studies continue confirmingan importance ofstudying morphological and functional aspects of the development of various medical complications in conditions of AHin the 21st century, thereby suggesting the relevance of such studies from the standpoint of both medical science and public health. The importance of discussed issues is due to the fact that nearly 17 million people worldwide die from cardiovascular diseases (CVD) annually; approximately 9.4 million of thosedie from complications of AH in the form of coronary artery disease and acute disorders of coronary and cerebral circulation [2]. Despite the positive dynamics in recent years, mortality from CVD in Russia remains one of the highest in the world, and our country ranks among the first in terms of the prevalence of AH (37.8%). Approximately 45% of deaths from heart disease and 51% from stroke are caused by elevated blood pressure (BP). It was confirmed that AH increases the risk of death from all causes by 2.2 times, and from CVD by 3.4 times.The main cause of death in people with high BP is CVD [3].

Currently, topical issues about the hereditary predisposition of more subtle trigger mechanisms for the development of AH and its complications are actively discussed in numerous publications. It is recognized that the regulatory functions of the body underAHconditions can be realized via the humoral pathway, through which, under the influence of various adverse factors, additional pathological processes are formed rather than compensatory responses alone. There is a point of view according to which some humoral mechanisms of AH formation may be associated with blood cells, cytochemical changes in which are detected earlier than their structural modifications.In the formation of humoral factors for the development of AH, a certain role belongs to leukocytes, among which a significant proportion are the most active blood cells known as polymorphonuclear leukocytes (PMN). We feel that one of the relevant aspectsis the investigations of the relationship between endothelial dysfunction developing in AH and changes in the metabolic profile of PMN. However, published data on changes in the nature of metabolic processes and functional activity of PMN in AH and its complications are extremely scarce [4,

5].Consequently, in our study, the main attention was focused on one of the poorly studied areas of hypertensiology: the search for a possible relationship between AH and metabolic disorders in blood leukocytes.

Analysis of the available information on the role of PMN in the formation of AH showed that they secrete plenty of granular enzymatic and nonenzymatic proteins with antibacterial, immunomodulatory and regulatory effects, as well as cytokines, chemokines, growth factors, oxygen-dependent and oxygen-independent biocidal factors. Hundreds of various receptor molecules are expressed on the surface membrane of PMN ensuring their communication with the microenvironment and other cells of the immune system. Changes in the functional state, in particular indicators of carbohydrate and lipid metabolisms and enzymatic activity of the main enzymes in PMN, may be markers of tissue damage in AH. PMN play a major role in the formation of leukocyte-endothelial interactions. The effects of migration, proliferation, aggregation and adhesion of PMN to the endothelial wall of blood vessels in target organs in conditions of AH are well known [6]. Activated PMN participate in oxidative stress in addition to exhibiting biocidal activity, inflammation and formation of the mechanisms of endothelial dysfunction. Particularly, they can damage surrounding tissues by releasing reactive oxygen species and can destroy nitric oxide, which is an endothelial vascular relaxation factor. For instance, the neutrophil-derived enzyme myeloperoxidase (MPO) with antimicrobial effect increases in the blood as a result of inflammation development in the walls of various types of vessels. The products of MPO catalysis are capable of interacting with glucose residues in intracellular polysaccharides and lipids, which causes the destruction of atherosclerotic deposits in the vascular wall and subsequent thrombosis. Therefore, the MPO level is a fairly accurate diagnostic marker of the risk of developing complications in case of AH. PMN, as well as a powerful inducer of oxidative stress in blood vessels, angiotensin II, promote the growth of smooth muscle cells, which can lead to hypertrophy and vascular stenosis. Thus, the degree of damage to the vascular wall in AH may be related to the dynamics of oxidative metabolic processes in neutrophils. That is why, currently, one of the promising directions of research includes examining possible changes in carbohydrate and lipid metabolisms, and activity of the main enzymes in blood PMNunderAH.

Based on the available data analysis regarding the participation of leukocytes in the formation of humoral mechanisms of the AH development, the goal of our study was to perform a comparative analysis of the characteristics of the intracellular metabolism in PMNof the peripheral blood in rats with inherited stress-induced arterial hypertension (ISIAH) vs. the normotensive animals (NTA).

Materials and Methods

As a model of AH, we used 15 intact sexually mature male rats representing the ISIAH line and weighing 250–300 g. These animals were characterized by consistently high BP levels and increased sensitivity to stress. The comparison group consisted of 20 nonlinear NTA. The animals were kept in standard vivarium conditions in accordance with the requirements of the 1985 International Guiding Principles for Biomedical Research Involving Animals adopted by the Council for International Organizations of Medical Sciences. Measurement of BP and heart rate (HR) was carried out in the tail artery of unanesthetized animals using an automated plethysmography with Natsume KN-209 electrosphygmomanometer (Japan). When studying heart sections, morphometric analysis was employed after staining with hematoxylin and eosin, and iron-hematoxylin according to Heidenhain. We then identified the mean diameter of cardiomyocytes, Kernohan index (the ratio of the vascular wall mesothelium thickness to the vascular lumen), and the mean number of functioning capillaries per unit area of the heart section.

After decapitation, blood was collected from the animals into tubes with heparin, which, compared with other anticoagulants, caused the minimum of morphological changes in leukocytes. Then smears were prepared and, after appropriate fixation, the content of lipids and glycogen in leukocytes, as well as the activity of various enzymes, were detected via histochemical methods [7]. Changes in the functional activity of leukocytes were recorded by the hematocrit (HCT) test (measuring the ability of neutrophils to generate reactive oxygen species). The amount of glycogen was identifiedby the McManus periodic acid Schiff (PAS) reaction. Another type of energy substrate (lipids), present in neutrophils in the form of drops of neutral fats, was stained with Sudan BlackB according to the method of Sheehan and Storey. Determination of lysosomal cationic proteins (LCP) was performed by the Pigarevsky's method. ATPase activity was detected via the Wachstein–Meisel procedure. The manifestation of succinate dehydrogenase (SDH) activity was identified using the R.P. Narcissov's modification of the method by M. Nachlas et al. (1960). An important and specific enzyme of redox processes in cells is MPO, the activity of which was determined by the Graham–Knoll method.

In each blood smear, we calculatedthe percentage of positively reacting cells, along with the mean cytochemical coefficient (MCC). The latter was calculated sensuAstaldi and Verga. The results of all cytochemical reactions were assessed by examining 100 granulocytes under high-poweredmicroscope. The grade of color intensity was determined in each cell. The absence of cytoplasmic staining in any reaction was taken as zero grade. Morphometric analysis was carried out on a BIOSCAN image analyzer.

Quantitative data were processed in MS Excel. To confirm the normality of the distribution, the Shapiro–Wilk test was employed. When describing the results of statistical data processing, we presented the mean value and its standard deviation. The significance of differences between two independent groups was determined using Student's t-test at an accepted significance level of 0.05.

Results

Prior to conducting hematological studies, we performed a comparative analysis of some indicators of the cardiovascular system condition in ISIAH ratsvs. NTA in order to confirm the morphological and functional changes characteristic of AH. We discovered that the difference in BPbetween ISIAH rats and NTA was 43%. E.g., BP was 113.4±7.3 mm Hgin NTAvs. 162.2±8.6 mm Hg in ISIAH rats ($p<0.001$). HR, equal to 488.0±12.0 beats/min in ISIAH rats, was 27% higher vs. NTA ($p<0.001$). At the same time, a

statistically significant increase in cardiac index and cardiomyocyte diameter was revealed in ISIAH rats (18.5±0.9 μm) vs.NTA (12.6±0.7 μm) ($p<0.001$). Hypertrophy of the wall of intramural arterioles was implied by a 46%-increase in the Kernohan index of ISIAH rats vs. NTA ($p<0.001$). When calculating the number of functioning capillaries per unit area of the myocardium section in NTA and ISIAH rats, we revealed a 22%-reduction in the density of the smallest vessels of the microvasculature in the latter. E.g., in AH, the number of functioning capillaries in the myocardium was 9.3±0.3 s.11.8±0.7 in animals with normal BP ($p<0.001$). Signs of dystrophic changes were observed in the endothelial lining of small vessels. Our data confirmed the fact that in adult ISIAH rats, along with elevated levels ofBP and HR, there were signs of myocardial hypertrophy, dystrophy of individual cardiomyocytes, and changes in the walls of small arteries of the heart; that is, there we observed morphological and functional disorders accompanying AH. Consequently, in the studied ISIAH rats, the correspondence of the most important morphological and functional parameters of the heart was impaired in the form of an increase in the volume of contractile cardiomyocytes and simultaneous rarefaction of the capillary bed in the myocardium, which, in turn, could lead to chronic hypoxia of this organ.

Due to the discovery of pronounced histological and functional signs of AH in ISIAH rats during this experimental study, more careful attention was paid to the analysis of the metabolic profile of leukocytes. Based on the circumstance that glycogen is one of the most readily available and labile cell products that plays an important role in the energy metabolism, its content in the cytoplasm of PMN of hypertensive animals was determined: specifically, we observeda 23%-decrease in the amount of glycogen compared with the control (1.56±0.07 in ISIAHvs.2.02±0.05 in NTA, $p<0.001$). However, no significant reduction in the number of cells containing glycogen was detected.

A comparative analysis of the MCC of lipids in neutrophils showed that there were no significant differences in ISIAH rats compared with NTA (1.83±0.07 in NTAvs. 1.86±0.09 in ISIAH, $p>0.05$). However, the number of leukocytes in which lipids were detected was slightly lower in ISIAH animals. In order to obtain more comprehensive information about the state (intensity) of metabolic processes in neutrophils during AH, we examinedthe activity of the most important enzymes. As cytochemical studies showed, the activity of intracellular ATPasewas reduced by 22% in ISIAH rats vs. NTA(1.53±0.08 vs. 1.96±0.04, $p<0.001$)in peripheral blood neutrophils against the background of AH, with a constant number of positively stained cells. MPO activity was suppressed to the same extent in neutrophils of ISIAH rats (1.60±0.03 vs. 2.05±0.05 in NTA, $p<0.001$). Along with a decrease in the enzymatic activity of ATPase and MPO, a slight increase (by 13%) in the activity of one of the redox enzymes, SDH, was detected in neutrophils (1.36±0.04 vs. 1.20±0.02 in NTA, $p<0.001$).

The presence of confirmed AH in animals did not affect the quantitative content of LCP in the cytoplasm of the PMN, judging by the MCC values (1.26±0.09 in ISIAH vs. 1.22±0.02in the control,$p>0.05$). At the same time, they exhibited an increase in the number of cells containing LCP (87.8±2.8 in the experiment vs. 77.0±0.8 in the control, $p<0.001$). A similar trend was revealed in the course of the HCT test, which reflected the degree of activation of the hexose monophosphate shunt function and the production of free radicals in the PMN. The number of cells with a positive reaction to the HCT test in ISIAH rats was 35% higher compared with this indicator in NTA (59.7±1.5 and 44.2±1.0, respectively, $p<0.001$). However, no significant differences were found in the degree of their saturation with diformazan grains (based onMCC values): 0.83±0.02 in NTA vs.0.85±0.04in ISIAH rats ($p>0.05$). Overall results reflecting the metabolic state of leukocytes in ISIAH rats based on MCC values are presented in the *Figure*.

Discussion

In previous experimental studies, we established that under the impact of pathological stress (T.P. Romanova's model), ISIAH rats (compared with NTA) developed more pronounced cardiac and cerebrovascular disorders in the form of large hemorrhagic foci in the brain and heart, along with permeability disorders of vascular walls with signs of endothelial dysfunction [8–11]. Taking into account previously obtained results, we paid close attention to changes in leukocyte metabolism in AH in this study. We based our assumptions on the following known facts: (1) blood cells and endothelial cells belong to two systems that are histogenetically and functionally closely interrelated, (2) both leukocytes and endothelial cells produce and secrete various compounds with both vasoconstrictor and vasodilator effects, and (3) endothelial cells and PMN are capable of exerting mutual modulating effects. For example, the effect of the vascular wall on the functional state of neutrophils can be realized through the metabolic products of arachidonic acid secreted by endothelial cells. In turn, leukocytes activated under the impact of pathogenic factors during degranulation can release substances that damage the endothelium and lead to increased permeability and saturation of the vascular wall with plasma, which can accelerate the formation of hemorrhagic foci. It is important to emphasize that both in a normal physiological state and during the development of pathological processes in the body, complex interactions of circulating blood cells and the vascular endothelium repetitivelyoccur. For example, the development of endothelial dysfunction may be facilitated by a decrease in the MPOactivity (and MPO is basically an antioxidant found in ISIAH rats). It is known that low concentrations of hypochlorite formed during MPO catalysis cause activation of endothelial cells accompanied by an increase in the expression of P-selectin and tissue factor, which leads to increased thrombogenicity of the endothelial surface. High concentrations of hypochlorite lead to apoptosis of endothelial cells. Besides, MPO can convert low-density lipoproteins into their atherogenic form easily captured by macrophages and high-density lipoproteins into a dysfunctional form, thereby disrupting the cellular transport of cholesterol. It is assumed that the described pattern allows MPO (both in its low and high concentrations) contributing to the formation and transformation of atherosclerotic plaques.

The detected inflated values of the HCT may imply signs of a metabolic burst, in which the number of released reactive oxygen species in leukocytes increases. This may result in the formation of endothelial dysfunction in ISIAH rats [12]. Reactive oxygen species, on the one hand, have biocidal properties; but, on the other hand, they can have an altering effect on the myocardium via free radical peroxidation of the intracellular structures in their own cells and tissues. LCP are also mediators of the biochemical phase of alteration. The increase in the number of cells containing LCP in ISIAH rats may be due to the fact that the substances they contain

participate (in conjunction with macrophages)in the formation of local protective function, causing the migration of leukocytes into the myocardium [13].

A change in the balance of energy-providing metabolites is also evidenced by the increase in the activity of SDH that catalyzes the oxidation of succinic acid (the most important source of energy replenishing the deficiency of energy-intensive molecules in myocardial lesions. At the same time, a decrease in the activity of intracellular ATPase (catalyzing the hydrolysis of ATP with the release of energy) was observed in leukocytes, which provides a trigger for the contraction of cardiomyocytes. Hence, in ISIAH rats, shifts in enzymatic processes occurring in the PMN (decrease in the activity of ATPase and MPO, which are ion-transporting enzymes of the heart, and a slight increase in the activity of SDH) are probably markers of the intensity of energy-supplying metabolic processes, which may represent a pathogenetic factor in the development of hypertrophic disorders in the myocardium with a simultaneous reduction in the number of functioningblood vessels.

We also noticed a a significant reduction in the content of the most labile and readily available polysaccharide, glycogen, in the PMN of ISIAH rats, which implied the tension of energy-supplying processes in the myocardium. It is likely that an increase in the functional activity of PMN occurs against the background of an increase in theirglycogencontent, while a decrease in the amount of cytoplasmic glycogen during glycogenolysis indicates an intensification of energy-dependent metabolic processes in leukocytes with subsequent inhibition of their functional metabolic activity. Some reduction in the number of PMN, in which lipids were detected, in the absence of changes in their MCC values, may indirectly indicate dyslipidemia and the involvement of leukocyte lipids in the pathogenesis of inherited stress-induced AH [15].

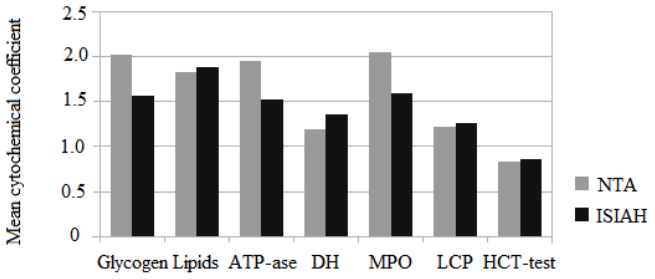

Figure. Metabolic indicators of polymorphonuclear leukocytes in normotensive and hypertensive rats using the mean cytochemical coefficient: *, statistical significance of differences between groups of normotensive animals (NTA) and rats with inherited stress-induced arterial hypertension (ISIAH) ($p<0.001$); SDH, succinate dehydrogenase; MPO, myeloperoxidase; HCT, hematocrit; LCP, lysosomal cationic proteins.

Our results not only implied the existence of a relationship between pathological processes in the myocardium and in leukocytes in the course ofAH. The revealed disorders of the cardiovascular system and intracellular metabolism of peripheral blood PMN in rats represent a complex of AH-induced changes in homeostasis, which once again confirm the polygenic nature of AH and the multiple phenotypic and functional manifestations of the disease. Perhaps, the emerging response of metabolic reactions of leukocytes leads to deeper myocardial damage [11]. Thus, it can be assumed that genetically determined metabolic dysfunction of peripheral blood PMNcan contribute to a decrease in the body's adaptive mechanisms and serve as one of the risk factors for the onset of AH and the development of its complications.

Our experimental study also showed that, due to a wide range of genetically determined phenotypic and functional manifestations of AH, including granulocytes and myocardium, ISIAH rats represent an effective model for studying the etiology and pathogenesis of AH. The results obtained in our study have diagnostic and prognostic significance when taking into account hereditary predisposition to AH and for the prevention of its complications [16]. As for clinical practice, our results also have a certain significance, since they can actualize the need for a full diagnosis and analysis of functional PMN lesions(besides solely quantitative disorders), and PMN are a mirror of homeostasis in AH. This is especially important for improving the treatment of AH, including vascularmetabolic therapy and immunotherapy. The latter statement is already used in the management of patients with AH, but requires expansion of the actual evidence base [17], which confirms the relevance of a further examination of the presentedproblem.

Conclusion

Evaluating the overall results of the comparative analysis, we can conclude that AH in ISIAH rats is accompanied by multidirectional genetically determined changes in metabolic processes of peripheral blood PMN, which, in turn, can cause or aggravate the development of endothelial dysfunction in blood vessels. Thus, the identified changes in the metabolic profile in blood leukocytes in the presence of AH may act as concomitant factors in the development of hypertrophic and dystrophic disorders of the myocardium, which ultimately contributes to a reduction in adaptive capabilities of the body and may serve as one of the trigger mechanisms for the development of deeper pathology underthe condition of AH. High rate of metabolic processes in blood leukocytes makes cytochemical studies particularly informative, allowing targeted observation of the pathological process dynamics and prediction of its outcomes.

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