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The Role Of The Cell Membranes In The Development Of Arterial Hyperetension And Drug Methods Of Its Correction.

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ABSTRACT

Nowadays, there is enough evidence for dysfunction of cell membranes (primarily transport of the cations, the regulation of the content of free intracellular calcium) as the cause of hypertension. The search of effective metabolic and antihypertensive drugs, which can effect on the functional state of the cell membranes, is particularly interesting. Hypertensive rats received 10 mg/kg/day amlodipine, 25 mg/kg/day bisoprolol and 1 mg/kg/day ellagic acid and combinations of these drugs via orogastric tube during three months. The study showed that erythrocyte hemolysis in hypertensive rats begins to manifest even in 0.5% and 0.45% solution of NaCl, while the erythrocytes of healthy rats under these conditions are not lysed. Amlodipine and bisoprolol are able to normalize the permeability of the membranes of red blood cells, but this parameter does not reach normal values. Ellagic acid has more pronounced membrane-protective properties. Concomitant use of amlodipine with ellagic acid is better restores the functional state of the erythrocyte membranes in hypertensive rats. Other combinations of the studied drugs are less effective. These findings correlate with our data that we obtained earlier in the study of myocardial ultrastructure and fatty acid composition of the cardiomyocytes in rats with hypertension.

Keywords: amlodipine; bisoprolol; ellagic acid; hypertension; erythrocytes.

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INTRODUCTION

For over a century hypertension remains a mystery. Researchers all the time face an insurmountable obstacle in explaining the immediate cause of the main manifestations of the disease - resistant elevation of blood pressure (BP).

Doubtless is the fact that the development of different theories has helped a lot in understanding of certain pathogenic mechanisms of hypertension. It has ensured effective antihypertensive therapy. However, the main and general goal of many studies is to identify the primary causes of hypertension was not achieved.

Nowadays, there is enough evidence for dysfunction of cell membranes (primarily transport of the cations, the regulation of the content of free intracellular calcium) as the cause of hypertension. One of the first works, which describes the abuse transmembrane ion transport in vascular smooth muscle cells in rats with spontaneous hypertension belong to Jones A.W. Other studies have found increasing number of Na^+ in the cells of the renal arteries of patients with hypertension [1]. This can be done by increased membrane permeability and increased passive diffusion of ions. The other type of transport function, which alters the intracellular concentration of Na^+, may be the reduction of the Na^+-K^+-ATPase activity. Also in the pathogenesis of hypertension may be involved Na^+-K^+-Cl exchange transport, Na^+-Li^+ exchange transport. It has been suggested that defects in this transport systems are genetically determined and this can be intermediate link combining primary genetic defect of function of the cell membranes with changes in vascular smooth muscle tone.

Increase of Na^+ in smooth muscle cells of vessels facilitates easier depolarization and Ca^{2+} entrance. Also increasing concentration of Na^+ in the cell activates Na^+-Ca^{2+}-contertranport that increases intracellular free Ca^{2+} in the cytoplasm. This is confirmed by studies conducted in cardiomyocytes and smooth muscle cells of vessels in the rats with arterial hypertension [2].

Other studies found defective binding and fixing of $Ca^{2+}$by various cellular structures of smooth muscle cells in patients with hypertension and hypertensive rats. Reduction of Ca^{2+}-bindingability appropriate cellular structures of smooth muscle cells is regarded as the cause of raising the level of free calcium in the cytoplasm of these cells and their hypertonicity [3]. Later, Schwartz A. et al. found a negative effect of myocardial ischemia on capturing and linking of calcium by sarcoplasmic reticulum. It increases level of calcium inside of the cardiomyocytes [4].

The data that indicates the existence of widespread damage to cell membranes during arterial hypertension is not limited by the contractile cells but also adipose tissue. This new analysis showed a significant increase of intracellular calcium in the adipocytes, reduced ability of cytoplasmic reticulum to absorb these ions and reduced calcium binding ability of the plasmatic membrane [5].

However, vascular smooth muscle cells, cardiomyocytes, adipocytes are difficult to obtain isolated with preserving functional activity. In addition, it is difficult to measure intracellular concentration of electrolytes in these types of the cells and avoid surrounding extracellular electrolytes error. Much attention is devoted to the study defects of electrolyte transport in erythrocytes. Many studies found the same type of change in the number of Na^+, K^+ and Ca^{2+}during hypertension not only in erythrocytes, but also in vascular smooth muscle cells [6].

The permeability of the cell membranes of erythrocytes may be analyzed by the measurement of osmotic erythrocyte resistance. This option is commonly used to assess the functional state of the cells and as informative test for the detection of even small initial disturbances in the body [7].

If the erythrocyte gets into hypotonic environment, water enters into the cell first (membrane permeability coefficient for water is 10 fold higher than for electrolytes) to equal osmotic pressure inside and outside the cell. Firstly, the swelling erythrocyte membrane area does not change, only cell volume increases. Upon reaching a spherical shape, further increase in volume without increasing the surface area of the cytoplasmic membrane becomes impossible - isotropic membrane stretching (stage 1). At a certain threshold level of tension lipid pores become energetically favorable - hemolytic pores (stage 2). These pores are fundamentally different from protein channels. Hemolytic pores unlike channels do not have selectivity, this

option depends on the size of the pores. Physical and mathematical calculations revealed that the lipid pores is affected by two antagonistic forces: the edge line tension of perimeter (promotes growth of pores), and phospholipid surface tension (causes compression of pores) [8]. Intracellular contents (potassium ions initially, later hemoglobin) flow out through hemolytic pores. The pressure inside the cell quickly drops to a critical value and relative cell volume decreases (stage 3). If the radius of pore curvature smaller than the critical radius, pore is closes because its existence is thermodynamically unfavorable. If the radius of curvature larger then pore critical radius, rupture will take place as a result of the unlimited growth of pores [9].

Cytoskeleton proteins allow erythrocytes to keep shape, and so full mechanical destruction of cells does not occur, but "shadows" of erythrocytes are formed (when erythrocyte hemoglobin leaving, optical density decreases and erythrocytes become almost invisible). If cytoskeleton is underdeveloped or it is suffered from damage, the mechanical strength of the cell will depend mainly on the size of lipid pores.

MATERIALS AND METHODS

We used pure substance of amlodipine (Glochem Industries Limited - India), bisoprolol (SIC "Borshchahivskiy CPP" - Ukraine), Ellagic acid (SIC "Borshchahivskiy CPP" - Ukraine).

White normotensive rats and rats ISIAH line (inherited stress-induced arterial hypertension) were both sexes [10].The animals were placed individually in special cages. All experimental animals were healthy. Rats were kept in a room with constant temperature (23-25 ° C) and sufficient natural light. Animals had free access to standard food diet and water. All experimental studies were conducted in compliance with ethical standards (Directive 86/609 / EEC) and GLP.

Experimental animals were divided into 9 groups (7 rats in each group): 2 control groups and 7 experimental. I control group – rats with normal blood pressure; II control group – rats with arterial hypertension; III group received 10 mg / kg of amlodipine; IV group – 25 mg / kg of bisoprolol; V group – 1 mg / kg of ellagic acid; VI-IXgroups received combinations of these drugs. The research lasted 3 months. Drugs were administered intragastrically once per day via orogastric flexible catheter. Drugs were administered sequentially with an interval of 30 minutes during combined use. Bearer for all drug substances was water. After enteral administration of these substances, animals were allowed food in 4 hours.

Study drugs were used in therapeutic doses calculated for rats.

Osmotic erythrocyte resistance(OER) was determined by the level of hemolysis of erythrocytes in a series of buffered hypotonic solutions of sodium chloride with concentration from 0.5% till 0.1% at pH 7.4 [11]. The degree of hemolysis was investigated in the supernatant after centrifugation by photoelectric colorimeter with the green photofilter (540 nm).

All statistical calculations were carried out using program "BioStat 2009". Differences were considered significant when the $p \leq 0,05$.

RESULTS

Rats with arterial hypertension (AH)have significant changes of the OER (Table 1). Thus, erythrocyte hemolysis begins to manifest even in 0.5% and 0.45% solution of NaCl (13,5 ± 3,2% and 30,9 ± 2,5%, respectively), while the erythrocytes of healthy rats under these conditions are not lysed. Significantly increasing hemolysis of the erythrocytes in 0.4% solution of NaCl (78,1 ± 6,1% as compared with 28,8 ± 4,1% in control I) and in 0.35% solution (93,0 ± 3,3% vs 74,0 ± 4,2% in control I). These changes may indicate a serious disruption of the functioning of the cell membranes, especially its permeability and ability to stretch.

During amlodipine administration OER increased in all investigated solutions of NaCl (Table 1). Compared to animals without treatment, percentage of hemolysis in 0.4% NaCl solution decreases from 78,1 ± 6,1% to 55,9 ± 2,9%. However, this figure does not reach control values of normotensive rats (55,9 ± 2,9% vs 28,8 ± 4,1%in control I). A similar pattern is observed when using bisoprolol, although it is less pronounced (in 0.4% NaCl solution lysis of erythrocytes is 60,1 ± 3,4% as compared with 78,1 ± 6,1%in control II). The

combined use of amlodipine with bisoprolol does not changeOER in contrast with amlodipine monotherapy (in 0.4% NaCl solution lysis of erythrocytes was 53,0 ± 2,2% to 55,9 ± 2,9%).

Table 1: The effect of amlodipine, bisoprolol, ellagic acid on osmotic resistance of red blood cells in rats with hypertension, n=7

Groupsofanimals	ConcentrationofNaClsolution(%)				
	0,5	0,45	0,4	0,35	0,1
	percentageoferythrocytehemolysis				
Normotensiverats	0	0	28,8±4,1	74,0±4,2	100,0±0
Hipertensiverats	13,5±3,2*	30,9±2,5*	78,1±6,1*	93,0±3,3*	100,0±0
Hipertensiverats + amlodipine	2,1±1,4*#	15,1±2,2*#	55,9±2,9*#	77,9±2,4#	100,0±0
Hipertensiverats + bisoprolol	7,1±2,1*#	21,9±4,1*#	60,1±3,4*#	81,1±4,8#	100,0±0
Hipertensiverats + amlodipine + bisoprolol	1,1±0,9*#	13,1±1,6*#	53,0±2,2*#	75,0±2,9#	100,0±0
Hipertensiverats + ellagic acid	0	0	41,1±3,8*#	68,1±5,8#	100,0±0
Hipertensiverats + amlodipine + ellagic acid	0	0	25,9±3,9#	56,2±6,0*#	100,0±0
Hipertensiverats + bisoprolol + ellagic acid	0	0	35,0±3,7#	60,9±2,3*#	100,0±0
Hipertensiverats + amlodipine + bisoprolol + ellagicacid	0	0	21,0±1,2*#	51,1±1,6*#	100,0±0

Notes: * –theprobabilityofgroup I (p≤0,05); # – theprobabilityofgroup II (p≤0,05).

Ellagic acid leads to a significant improvement of OER. Thus, in 0.5% and 0.45% NaCl solution erythrocyte hemolysis is not observed, that is the same as in the rats with normal blood pressure. In 0.4% NaCl solution level of hemolysis of erythrocytes is reduced from 78,1 ± 6,1% to 41,1 ± 3,8% compared with hypertensive rats, and in 0.35% solution there is no difference from normotensive animals (68,1 ± 5,8% to 74,0 ± 4,2%). During combined use of amlodipine with ellagic acid is observed stabilization permeability (osmotic resistance of the erythrocytes restored to normal level). Effect of combination of bisoprolol with ellagic acid on the degree of lysis is not significantly different from the effect of ellagic acid in monotherapy. Triple therapy the most influences on the OER in rats with hypertension, increases it even compared to normotensive rats.

DISCUSSION

The study of the state of erythrocyte membranes in hypertensive rats established serious violations of their functions, especially permeability and ability to stretch. This is evidenced by a significant increase in the proportion of lysed erythrocytes in all investigated concentrations of hypotonic solution of NaCl.

Amlodipine and bisoprolol are able to normalize the permeability of membranes of the erythrocytes, but this option does not reach normal values. Ellagic acid has expressed membrane-protective properties, that also was found in the joint administration with amlodipine or with bisoprolol.

These findings correlate with our data that we obtained earlier in the study of myocardial ultrastructure and fatty acid composition of the cardiomyocytes in rats with hypertension [12, 13].

The positive effect of amlodipine on the state of the membranes ,we explain due to its ability to block excessive flow of calcium ions into cells. As a result, amlodipine prevents phosphorylation of proteins of the cytoskeleton of erythrocytes and preventscontractile activation of actomyosine complex. Amlodipinealso reduces the activity of phospholipase A_2, thereby reduces excessive destruction of phospholipids of the membranes [14].

Bisoprolol's effect on the membrane of erythrocytes can not be explained by changes in membrane'sfluidity. Although we can not exclude a direct effect of bisoprolol on physico-chemical properties of the membrane. The ratio of the lipophilicity (log P = 2,2) indicates that bisoprolol hasability to penetrate cell membranes by passive diffusion. Consequently, one of the possible mechanism for reducing erythrocyte

hemolysis by bisoprolol is direct stimulation of reparation of lipid pores.Similar results were obtained in the study influence of nonselective β-blockers timolol and propranolol on fresh human erythrocytes during hypotonic hemolysis [15].

Membrane-protective abilities of ellagic acid are due to its antiradical effects.Ellagic acid has phenolic hydroxyl groups and the stable structure.Ellagic acid inactivates nonenzymic lipid peroxidation, inhibitsxanthine oxidase. Ellagic acid can defendantioxidant system (catalase, glutathione reductase, glucose-6-phosphate dehydrogenase) due to ellagic acid's mobile hydrogen atoms act as a donors for hydrogen-dependent NADPH enzymes.

Also ellagic acid as polyphenolic compound has a certain hydrophobicity, which depends on the number of aromatic rings in its structure.So ellagic acid can incorporate in a hydrophobic phase of the membranes. This facilitates direct impact on the structural and dynamical state of the cell membranes; allows ellagic acid function as scavenger of active oxygen forms and interrupt free radical's damageof the membranes [16].Similar results were obtained while studying the action of nonsteroidal antiinflammatory drugs and flavonoids [17].

Small difference combined administration of amlodipine, bisoprolol and ellagic acid compared with monotherapy with these drugs may be explained by the fact that many compounds with different chemical structure may exhibit their protective effects through nonspecific competitive interactions with components of hemolytic pores [15].

In conclusion, we can say that the pathogenesis of hypertension are not yet fully understood. The search of primary hypertension genetic markers is not finished yet and treatment of this disease requires finding new efficient and reliable medicines.

CONCLUSION

- Amlodipine and bisoprolol are able to normalize the permeability of the membranes of red blood cells, but this parameter does not reach normal values. Ellagic acid has more pronounced membrane-protective properties.

- Concomitant use of amlodipine with ellagic acid is better restores the functional state of the erythrocyte membranes in hypertensive rats. Other combinations of the studied drugs are less effective.

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