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Pharmacological Correction of Endothelial Dysfunction in Rats using e-NOS Cofactors.

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ABSRACT

Investigation of endotelioprotective effects of tetrahydrobiopterin, L-norvaline, L-arginine, and combinations thereof in modeling L-NAME induced endothelial dysfunction. In the combined use of e-NOS cofactors shown positive pharmacodynamic interaction and increase the effectiveness of therapy compared to administration of the individual compounds.

Keywords: endothelial dysfunction, L-NAME, ADMA, Tetrahydrobiopterin, L-arginine, L-norvaline.

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INTRODUCTION

Biopterin metabolism is critical for the regulation of NOS activity. It has been suggested that depletion of BH_4 and reduction in the BH_4/7,8-BH_2 ratio are critical for the regulation of endothelial production of O_2^- as well as NO [1, 2].

Tetrahydrobiopterin is one of the most potent naturally occurring reducing agents and an essential cofactor required for enzymatic activity of nitric oxide synthase (NOS). The exact role of tetrahydrobiopterin in the control of NOS catalytic activity is not completely understood.

Tetrahydrobiopterin is a cofactor essential for the catalytic activity of all three NOS isoforms [3, 4, 5]. During the last 10 years, significant progress has been made in understanding the role of tetrahydrobiopterin in the control of NOS function.

In the early 1990s biochemical studies demonstrated that in the presence of suboptimal concentrations of tetrahydrobiopterin, activation of nNOS leads to "uncoupling of NOS" with subsequent increased formation of superoxide anions and hydrogen peroxide [6, 7]. These findings have been confirmed and extended to eNOS [9, 10], suggesting that in endothelial cells consumption of NADPH can become uncoupled from nitric oxide synthesis, resulting in the production of superoxide anions and hydrogen peroxide. It is important to note that a series of in vitro biochemical studies demonstrated that eNOS is the most "tightly coupled" of the NOS isoforms [8], implying that nNOS and iNOS are potentially more powerful sources of reactive oxygen species.

Consequently, a strategy aimed at protecting the endogenous BH4 from oxidation attack by peroxynitrite can lead to preservation of optimal concentrations of endothelial tetrahydrobiopterin.

Moreover, the limitation of nitric oxide may be due to the high activity of arginase - degrading enzyme L-arginine in the mucosa of the small intestine. It is evident that the suppression of the high arginase activity to reduce the risk and incidence of heart disease and blood vessels. For this purpose it is possible to use inhibitors of arginase. Among this group of substances of greatest interest L-norvaline - non-selective inhibitors of the enzyme arginase (DLCostanzo, M. Ilies, 2010).

The present study investigated the possibility of pharmacological correction of endothelial dysfunction in rats using tetrahydrobiopterin, L-arginine, L-norvaline, and there combinations.

PROCEDURE

The experiments were performed on the white male Wistar rats weighing 200-250 g.

NO-synthase blocker N-nitro-L-arginine methyl aether (L-NAME) was inducted intraperitoneally (i.p.) in a dose of 25 mg/kg/day, once a day within 7 days.

Tetrahydrobiopterin was given intragastrical in a dose 10 mg/kg, L-norvaline was given i.p. in a dose of 10 mg/kg, L-arginine was given intragastrical in a dose 200 mg/kg. All substances were administered once a day within 7 days.

The animals were divided into groups (n = 10): 1 - control; 2 – L-NAME 25 mg/kg; 3 - L-NAME 25 g/kg + tetrahydrobiopterin (BH4) 10 mg / kg; 4 - L-NAME 25 g/kg + L-arginine 200 mg / kg; 5 - L-NAME 25 g/kg + L-norvaline 10 mg / kg; 6 - L-NAME 25 g/kg + BH4 10 mg / kg + L-arginine 200 mg / kg; 7 - L-NAME 25 g/kg + L-arginine 200 mg / kg + L-norvaline 10 mg / kg.

On the day 8 from an initiation of experiments under anaesthetic (chloral hydrate 300 mg/kg) a catheter in the left carotid artery for recording of indexes of blood pressure (BP) was entered. Bolus introduction of pharmacological agents was made into a femoral vein. Hemodynamic indexes: the systolic arterial pressure (SAP), a diastolic arterial pressure (DAP) and cardiac contractions rate metered continuously with a hardware-software complex «Biopac». Besides BP measuring a series of the functional trials was led in introduced succession: 1. endothelium dependent vasorelaxation test (intravenous entering of a solution of acetylcholinum

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(AH) in a dose of 40 mkg/kg); 2. endothelium independent vasorelaxation test (intravenous entering of a solution of sodium nitroprussidum (NP) in a dose of 30 mkg/kg) [11, 12, 13].

Level of endothelial dysfunction at the experimental animals, and also a level of its correction by researched drugs valued on coefficient of endothelial dysfunction (CED). This coefficient settled up by formula: CED = SBP_{NP}/SBP_{AH} where SBP_{NP} – the area of triangle above a BP recovery curve at a functional test with NP entering, SBP_{AH} – the area of triangle above a BP recovery curve at a functional test with AH entering. Points of a smaller cathetus of this triangle are the points of BP before the test and a point of maximum reduction of a BP, and the bigger cathetus is a time of BP restoration [11, 12, 13] (figure 1).

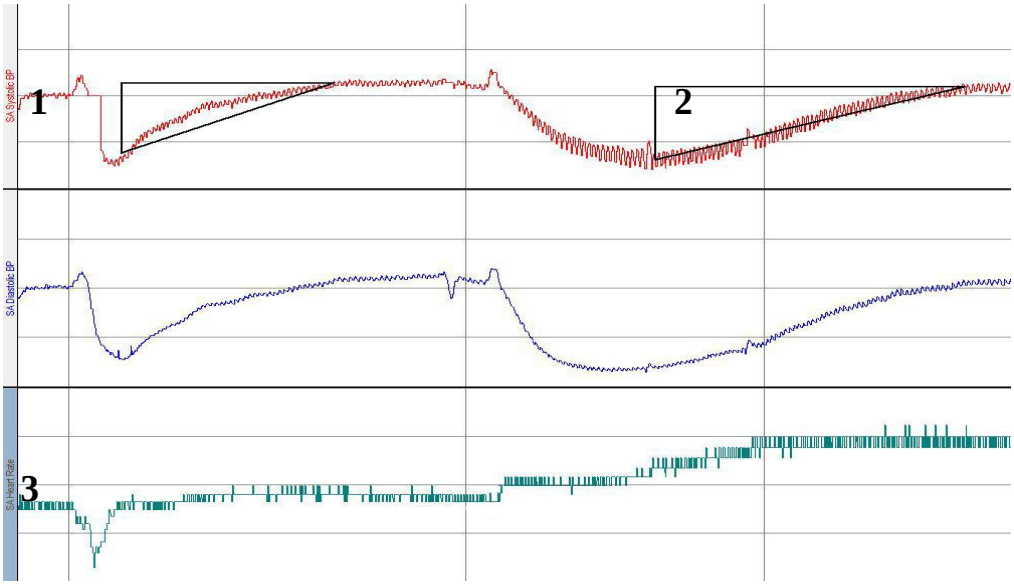

Figure 1: The dynamics of systolic and diastolic blood pressure and heart rate during the functional vascular trial with intravenous solutions of acetylcholine and sodium nitroprusside.

Note. 1 - Triangle of recovery curve in blood pressure in response to acetylcholine; 2 - triangle on the recovery curve of blood pressure in response to sodium nitroprusside; 3 - the end of the cardiac component and stabilization of heart rate.

The results were expressed as the mean (M) ± the standard error of mean (m). Differences were considered significant at $p < 0.05$.

FINDINGS OF THE STUDY

Effect of test substances on the baseline blood pressure in anesthetized rats modeling with L-NAME induced endothelial dysfunction are shown in Table 1. Intraperitoneal administration of L-NAME caused a statistically significant increase in blood pressure. It found that tetrahydrobiopterin, L-arginine and L-norvaline as monotherapy had no effect on baseline blood pressure (Table 1, SBP, DBP).

When using a combination of tetrahydrobiopterin with L-arginine and L-arginine with L-norvaline statistically significant decrease in blood pressure ($p < 0.05$), most marked in the group L-Arginine + L-norvaline (Table 1, SBP, DBP).

When calculating the coefficient of endothelial dysfunction found that BH4 at a dose of 10 mg / kg, L-norvaline 10 mg / kg, and L-arginine, 200 mg / kg as a monotherapy had endotelioprotektive action, expressed in reducing the coefficient of endothelial dysfunction. Combined use of tetrahydrobiopterin with L-arginine and L-norvaline with L-arginine led to an optimal reduction factor of endothelial dysfunction, which values in these groups were approaching that of an intact animal groups (Table. 1 CED).

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Table 1: Effect of tetrahydrobiopterin, L-arginine, L-norvaline, and there combinations to the functional indicators of endothelial dysfunction

Animal groups	SBP, mmHg.	DBP, mmHg.	CED, conv.
Control	137,7 ±3,7	101,9 ± 4,3	1,1±0,1
L-NAME	190,3 ± 6,7*	145,0 ± 3,9*	5,4±0,6*
L-NAME + BH4	170,3±6,7*	128,8±5,2**	2,8±0,4**
L-NAME + L-arginine	177,6 ± 9,6*	140,1 ± 6,4*	2,5±0,1**
L-NAME + L-norvaline	180 ± 4,7	144,6±10,2	2,1±0,2**
L-NAME + BH4 + L-arginine	149,1±4,1**	112,4±5,1**	1,6±0,3**
L-NAME + L-arginine + L-norvaline	135,6±10,1**	102,5±5,8**	1,7±0,2**

Note: SBP, DBP - systolic and diastolic blood pressure; CED - the coefficient of endothelial dysfunction; * - P <0.05 compared to the control group; **- P <0.05 compared to L-NAME group of animals.

In the analysis of absolute values of area above the curve of blood pressure recovery during the sampling optimal ratio endothelium-dependent and endothelium independent vasodilatation (close to a series of control animals) showed groups of animals treated tetrahydrobiopterin with L-arginine and L-arginine with L-norvaline. Since the area of vascular response in response to intravenous acetylcholine in the group of animals treated with BH4 + L-arginine reached 1439,1 ± 144,6 conv, L-arginine + L-norvaline - 885,4 ± 13,1 conv. u (In the control 1268,0 ± 74,8), in response to the intravenous injection of sodium nitroprusside - 2302,6 ± 156,9 and 1681,3 ± 151,4 conv. respectively (in the control - 1375,3 ± 93,7). In addition, the decrease in CED in the group of animals treated with L-arginine and tetrahydrobiopterin as monotherapy, was due to a statistically significant reduction in the area of endothelium dependent reaction, but the area of endothelium independent reaction was not significantly changed. This fact indicates a pronounced effect of BH4 and L-arginine in the endothelial cells and the synthesis of nitric oxide system.

Thus, the results of our studies have confirmed that the presence of BH4 is necessary but not sufficient to recover nitric oxide metabolism and activity of endothelial NO-synthase (eNOS) for modeling endothelial dysfunction. Implementation Mechanisms obtained effects of L-arginine, considering the above, apparently due to the fact that L-arginine increases the NO synthesis in endothelial cells by competitive displacement of inhibitors of an enzyme eNOS [14]. The mechanism of action of L-norvaline due to its structural similarity to ornithine, which is one of the metabolic products of the urea cycle. Mediated effect on L-norvaline arginase activity is associated with inhibition ornitintranskarbamilazy which catalyzes the conversion of ornithine to citrulline urea cycle.

CONCLUSION

Tetrahydrobiopterin 10 mg / kg, L-norvaline 10 mg / kg of L-arginine, 200 mg / kg exhibited a pronounced effect in modeling endotelioprotektivnoe L-NAME induced endothelial dysfunction. The most effective reduction in data analysis QED monotherapy observed in the group of animals treated with L-norvaline 10 mg / kg.

In the combined use of L-arginine to L-norvaline and L-arginine with tetrahydrobiopterin found positive pharmacodynamic interaction that resulted in a statistically significant reduction in systolic and diastolic blood pressure and reduce the maximum rate endotelailnoy dysfunction.

Require further study approaches to pharmacological correction of endothelial dysfunction with the use of drugs with different mechanisms of action have different points of application in the realization of its endotelio- and cardioprotective effects. Of particular interest is the combined use of tetrahydrobiopterin as a preparation for overcoming the eNOS uncoupling and traditional antihypertensive therapy with proven endotelioprotektive properties.

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