Hemodynamic Effects of Peptide Fragments of Differentiation Factor HLDF

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Hemodynamic activity of peptides from differentiation factor HLDF (promyelocytic HL-60 line) was studied on WKY and SHR-SP rats. Intravenous infusion of the test peptides was accompanied by changes in blood pressure and heart rate, which depended on the structure of peptides and functional activity of the organism and differed in normotensive and hypertensive animals.

Key Words: *differentiation factor HLDF; hemodynamics; rats*

Mechanisms and functional role of differentiation and apoptosis in cells are intensively studied in modern biomedicine. Much attention is given to regulatory factors providing integrative functions of the nervous, hormonal, and cardiovascular system. These factors not only inhibit proliferation, but also activate programmed cell death. One of these factors is human leukemia differentiation factor (HLDF). HLDF is a protein factor (Table 1) isolated from the culture medium of transretinoic acid-induced human promyelocytic leukemia HL-60 cells. HLDF inhibits proliferation of HL-60 cells and stimulates their proliferation into granulocytes [4,10]. This protein was found in the blood and nervous system of mammals [14]. This factor contained two active fragments: hexapeptide HLDF-6 possessing differentiation capacity of the full-length factor and exhibiting protective activity [1,3,5,6,13] and octapeptide HLDF-8 exhibiting apoptogenic activity [2].

The dynamics of HLDF concentration in the blood and cerebrospinal fluid was evaluated in patients with acute stroke. It was found that the concentration of this protein significantly decreases in the blood, but remains unchanged in the cerebrospinal fluid over the 1st day of stroke [14]. These data suggest that regulators of apoptosis are involved in the pathogenesis of cerebral ischemia and, probably, possess cardiovascular activity. Published data show that apoptosis in cardiomyocytes after cardiac injury (*e.g.*, myocardial ischemia and infarction) contributes to heart failure [7-9]. The factors that regulate apoptosis in cells and modulate function of the heart and vessels are of particular importance in this respect. Here we studied the effects of HLDF and peptide fragments HLDF-6, HLDF-8, HLDF-14, and HLDF-24 on cardiovascular function in normotensive rats and hypertensive animals predisposed to the development of stroke.

MATERIALS AND METHODS

Experiments were performed on male normotensive WKY rats and male hypertensive SHR-SP rats weighing 300-350 g. The animals were obtained from the nursery of laboratory animals (Branch of the Institute of Bioorganic Chemistry). The rats were maintained at 18-24°C, 30-70% humidity, and 12:12-h light/dark regimen under standard condi-

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TABLE 1. Differentiation Factor HLDF and Its Peptides

Amino acid sequence and peptide position in the factor	Peptide name
^{1}AGLMASLKLMLSAGPFVGWVSQMIPFSDWPRRWHRLKELLTGENHRCGIFVINK^{54}	HLDF
^{31}RRWHRLKELLTGENHRCGIFVINK^{54}	HLDF-24
^{41}TGENHRCGIFVINK^{54}	HLDF-14
^{41}TGENHR^{46}	HLDF-6
^{31}RRWHRLKE^{39}	HLDF-8

tions. They received a standard diet and water *ad libitum*. Experimental manipulations were performed according to the Institute Program of Humane Attitude to Laboratory Animals.

Polyethylene catheters were implanted 1 day before the experiment. One catheter was introduced through the femoral artery into the abdominal aorta for recording of blood pressure (BP) and heart rate (HR). Another catheter was inserted into the femoral vein for intravenous infusion of the test compounds (100 μl/kg). Peptides in various concentrations were dissolved in physiological saline. Nω-Nitro-L-arginine methyl ester (L-NAME, 2.5 mg/kg) was used as a NO-synthase blocker. BP and HR were measured by the direct method using an electromanometer. The data were processed on a computer.

The results were analyzed by Student's *t* test and Duncan's test (two-factor ANOVA).

RESULTS

Peptides from the differentiation factor of HL-60 leukocytes exhibited hemodynamic activity. Intravenous infusion of synthetic full-length factor HLDF (54 amino acid residues) in a dose of 0.2 μmol/kg did not change BP and HR in normotensive animals, while HLDF-24 in the same dose caused hypotension and tachycardia. BP decreased by 50±5 mm Hg, while HR increased by 118.9±22.4 bpm (Fig. 1). The degree of hypotension depended on the dose of HLDF-24. HLDF-24 in doses of 0.1, 0.8, and 1.7 μmol/kg decreased BP by 17.7±3.6, 63.2±6.7, and 75.1±15.4 mm Hg, respectively. The decrease in BP was accompanied by an increase in HR by 54.6±15.4, 182.0±13.8, and 169.1±14.1 bpm, respectively. Increasing the dose of HLDF-24 to 1.7 μmol/kg was followed by a short-term hypertensive phase (pre-hypotension period). A similar hypertensive reaction was observed after treatment with HLDF-8 in a dose of 1.7 μmol/kg. This peptide in a lower dose was ineffective (Fig. 2). Since HLDF-8 is a fragment of HLDF-24, it can be hypothesized that short-term hypertensive phase observed after HLDF-24 administration is related to the presence of HDLF-8. HLDF-6 and HLDF-14 in various doses were ineffective.

HLDF-24 in a dose of 0.2 μmol/kg produced various changes in mean BP and HR of normoten-

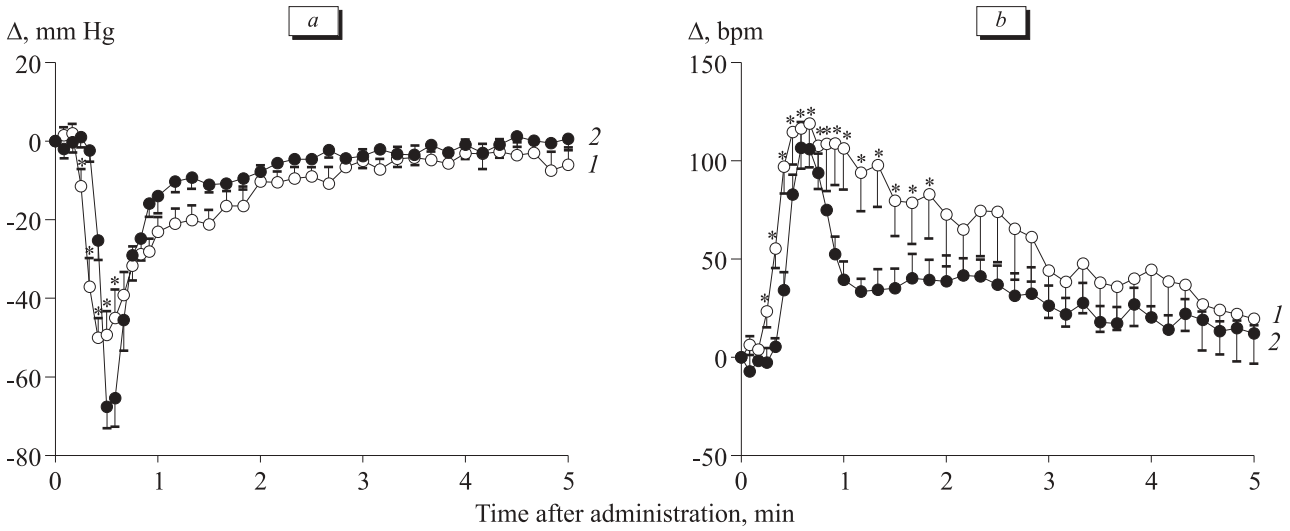

Fig. 1. Changes in the mean blood pressure (*a*) and HR (*b*) in normotensive (*1*) and hypertensive rats (*2*) after administration of HLDF-24 in a dose of 0.2 μmol/kg. Here and in Fig. 2: **p*<0.05 compared to *2*.

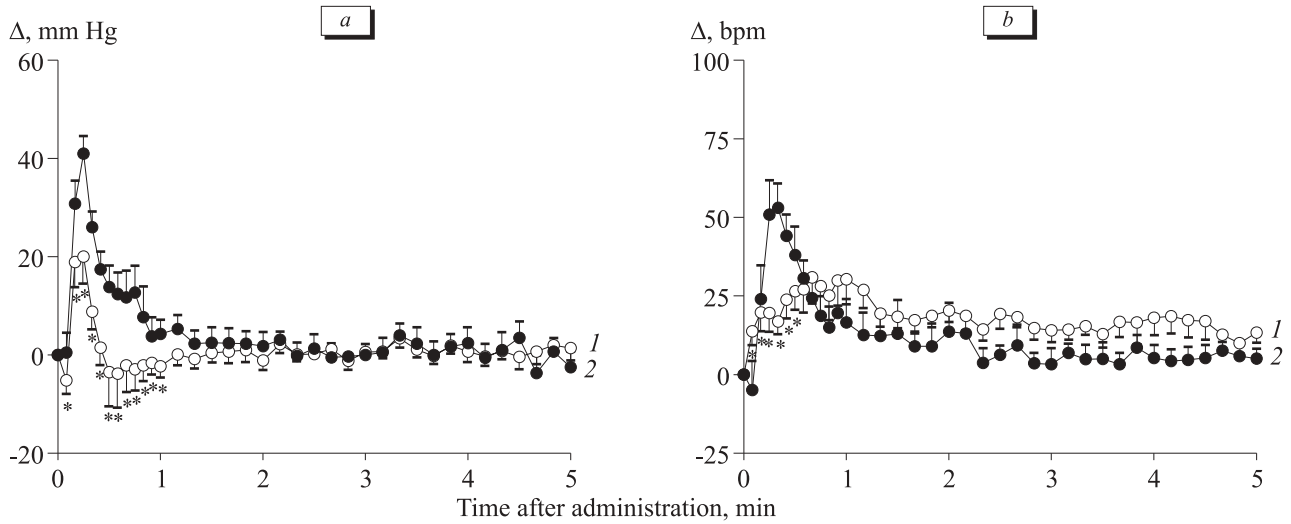

Fig. 2. Changes in the mean blood pressure (*a*) and HR (*b*) in normotensive (*1*) and hypertensive rats (*2*) after administration of HLDF-8 in a dose of 1.7 μmol/kg.

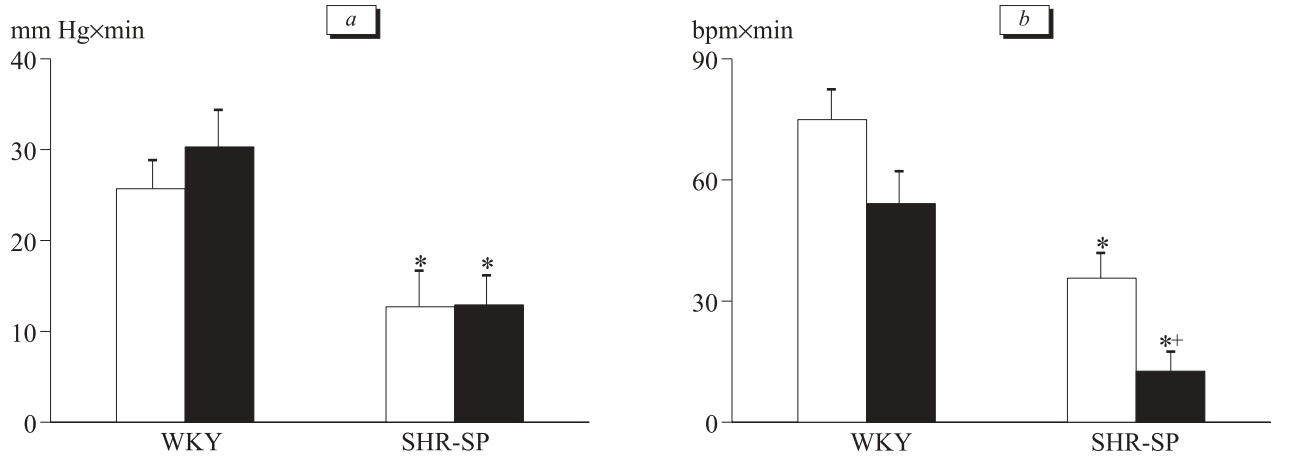

Fig. 3. Effects of HLDF-24 peptide in a dose of 0.2 μmol/kg (area under curve) on the mean blood pressure (*a*) and HR (*b*) in normotensive WKY rats and hypertensive SHR-SP rats not receiving (light bars) or receiving L-NAME (dark bars). **p*<0.05 compared to WKY; +*p*<0.05 compared to rats not receiving L-NAME.

sive and hypertensive animals (Fig. 1). For example, hypotension in hypertensive SHR-SP rats was more pronounced than in normotensive WKY rats (BP decrease by 67.6±5.3 and 50.0±5.0 mm Hg, respectively). However, the severity of tachycardia was similar in hypertensive SHR-SP rats and normotensive WKY rats (106.6±11.5 and 118.9± 22.4 bpm, respectively). The duration of tachycardia in hypertensive rats was lower than in normotensive animals. The differences between SHR-SP and WKY rats were also revealed during calculation of area under curve (47.0±5.3 and 76.2±13.0, respectively).

HLDF-8 in a dose of 1.7 μmol/kg produced a biphasic response in normotensive rats (Fig. 2). A short-term hypotensive response (-5.2±2.5 mm Hg) was followed by long-term hypertension (20.0±5.3 mm Hg). However, hypertensive animals exhibited only the increase in BP by 41.0±3.5 mm Hg. HR in hypertensive SHR-SP rats changed more significantly than in normotensive WKY rats (53.0±7.4 and 30.9±7.8 bpm, respectively). As differentiated from HLDF-24, the increase in BP was not accompanied by baroreflex-mediated decrease in HR under these conditions. We revealed the increase in HR, which is typical of the stress response.

The differences between normotensive and hypertensive rats persisted during NO-synthase blockade (Fig. 3). HLDF-24 produced similar changes in BP in control animals and L-NAME-treated rats. After administration of the test peptide, the degree of tachycardia in L-NAME-receiving rats was lower

than in control animals. These differences were statistically significant only in hypertensive rats. It can be hypothesized that the effects of HLDF-24 are mediated by the NO-independent mechanisms. Partial prevention of tachycardia is probably related to blockade of neuronal NO synthase, which decreases the reflex influence on the heart under conditions of peptide-induced hypotension.

Hemodynamic activity of HLDF fragments is probably mediated by several mechanisms. HLDF is structurally homologous to endothelin-converting enzyme (ECE). ECE antagonists produce hypotensive effects [12]. HLDF-24 probably acts as an ECE antagonist and suppresses the formation of endogenous endothelin. HLDF-8 exhibits DNA/RNA-hydrolyzing activity, which promotes free radical generation in the vascular bed [2]. Free radicals inactivate various enzymes, including Zn-metalloproteinases (*e.g.*, ECE). Free radicals also inactivate NO, which serves as a major vasodilatory compound in the vascular system [11].

Our results indicate that HLDF peptides have hemodynamic activity. This activity depends on the structure of peptides and functional activity of the organism and differs in normotensive and hypertensive animals. The mechanisms for hemodynamic activity of these peptides require further investigations.

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