Relationship Between Therapeutic Effect of the Peptide Preparation Semax and Severity of Brain Ischemia

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A decreases in local blood flow to the brain cortex within the first 15 min of bilateral occlusion of common carotid arteries determines the dynamics of brain blood flow and survival of rats. The effectiveness of the peptide preparation semax (an adrenocorticotropic hormone fragment 4-7) depends on the decrease in brain blood flow within the first 15 min of occlusion, reaching the maximum in severe brain ischemia.

Key Words: *brain ischemia; blood flow; semax*

Wide application of peptides in clinical practice has been hampered by "ambiguity" of their effects due to different receptor binding in different organs, heterogeneity of receptors [10], and conformational modifications [11]. Peptides not only modulate the effects of neurotransmitters, hormones, and other physiologically active substances [2,7,12], but also change their properties and activity under the action of catecholamines [8], histamine, heparin [3], etc. Since any disease is accompanied by alterations of humoral, cellular, and tissue homeostasis that reflect stages and severity of pathology, quantitative and qualitative changes in the spectrum of biologically active substances in the organism may determine selective activity of peptides. In order to check up this hypothesis, we examined the effect of the peptide preparation semax on local blood flow in the brain in ischemia of varied severity.

MATERIALS AND METHODS

Experiments were carried out on 53 male outbred albino rats (body weight 250-350 g) under chloral hydrate anesthesia (0.6 g/kg intramuscularly). Brain ischemia was produced by bilateral occlusion of common carotid arteries. Local brain blood flow (BBF) was measured by hydrogen clearance in the sensorimotor cortex for 1 h before and 3 h after brain ischemia using an ITK-2M device. In order to evaluate the effect of semax under most unfavorable conditions, we used rats with a low BBF (<60 ml/100 g×min). Semax (Met-Glu-His-Phe-Pro-Gly-Pro) is a 4-7 fragment of adrenocorticotropic hormone with hydrophobic radicals at the C-terminal (Pro-Gly-Pro), which increases its stability, effectiveness, and ability to cross the blood-brain barrier [5]. Semax was synthesized at the Laboratory of Regulatory Peptides (Institute of Molecular Genetics, Russian Academy of Sciences). Semax applied onto mesenteric lymph vessels (LV) in a dose of 0.0025-200 μg/kg in 0.1 ml normal saline and injected intraperitoneally in a dose of 50, 150 and 200 μg/kg×ml 15 min before occlusion of carotid arteries. Microcirculation rate in the mesenteric LV was studied by biomicroscopy. The results were analyzed using Student's *t* test and correlation coefficients.

RESULTS

Application of semax (0.0025-200 μg/kg) onto mesenteric LV had no effect on the contractile activity of LV wall and valves. However, it caused a transient (2-3 min) pendulum-like movement of lymph in the centripetal direction. An increase in semax dose did

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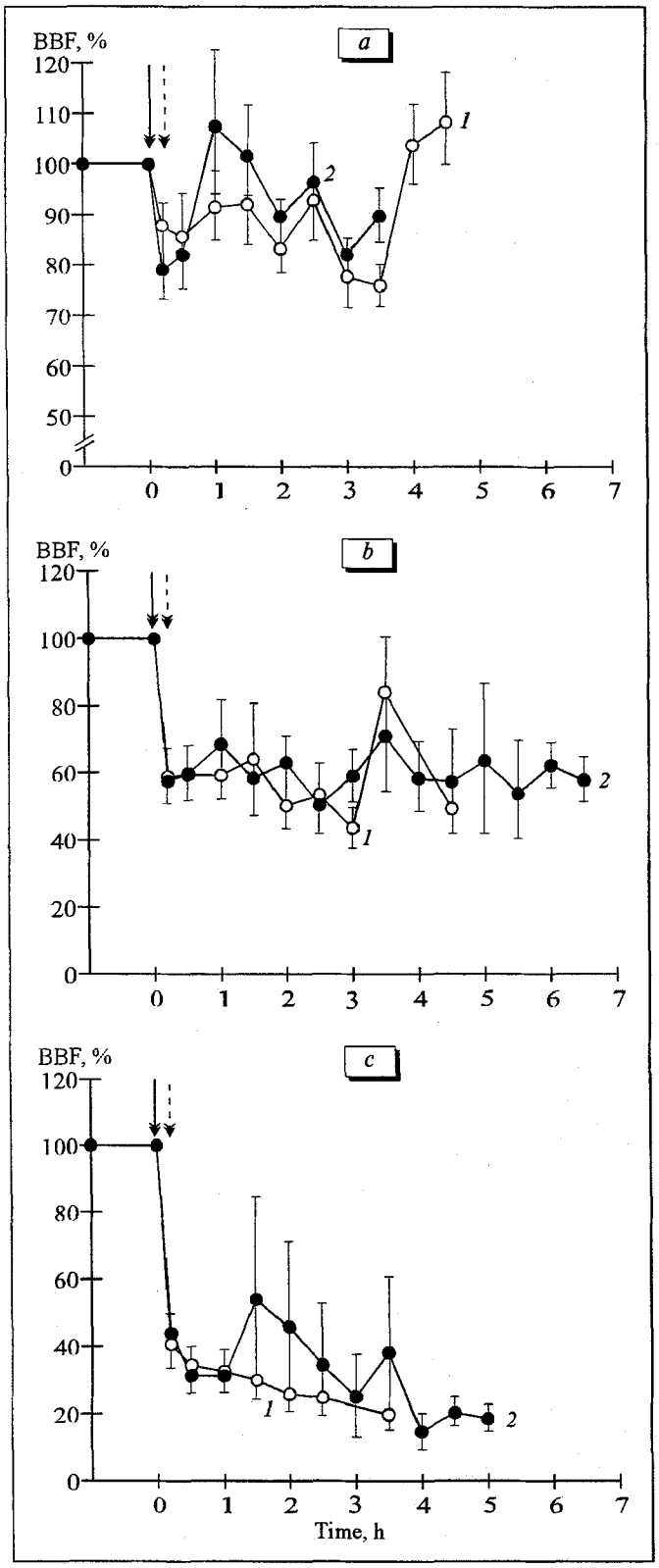

Fig. 1. Effect of the oligopeptide semax (150 μg/kg) on local blood flow in brain cortex (BBF) upon bilateral occlusion of the common carotid arteries. *a*) mild ischemia (BBF>70%); *b*) moderate ischemia (BBF=50-70%); *c*) severe ischemia (BBF<50%). *1*) control; 2) intraperitoneal administration of semax. Solid arrow indicates the beginning of occlusion. Broken arrow indicates administration of semax.

not change the response of blood and lymph microvessels.

In control experiments, cortical BBF was 35.01± 2.52 ml/100 g×min. Variations of this parameter during a 3.5-h observation period were not higher than 90-115% of the mean initial level within 40 min of observation, which was taken as 100%.

Intraperitoneal injection of 0.14 M NaCl (1 ml) induced no statistically significant changes in BBF compared with the control ($p>0.05$).

Bilateral occlusion of the common carotid arteries reduced BBF by 40% compared with the initial level. Intraperitoneal injection of semax (50, 150, and 200 μg/kg in 1 ml solution) 15 min after carotid artery occlusion (CAO) caused transient opposite changes in BBF, which did not allow us to make any conclusion about its effectiveness. Special attention was paid to different reduction in BBF within the first 15 min of occlusion (prior to administration of semax): in 23% of rats it decreased by >30% and in 30% of rats by >50%. In most animals (47%) a moderate (30-50%) decrease in BBF was observed. According to these data, the rats were divided into 3 groups: group 1 consisted of rats with mild brain ischemia (BBF>70% of the initial level before CAO taken as 100%). Group 2 included rats with moderate ischemia (BBF=50-70%). Severe brain ischemia (BFF<50%) occurred in group 3 rats. Statistical analysis of the BBF values before and after arterial occlusion (Table 1) showed that the decrease in BBF is the highest in rats with the highest initial BBF. The lowest decrease in BBF was observed in rats with initially low BBF. Although the resistance of rats with different BBF to the standard brain ischemia is different, BBF after CAO is similar. The strongest correlation (r=-0.62) between BBF before and after occlusion was established in rats with severe ischemia. The correctness of our classification of the rats was confirmed by their survival within a 3-h period after CAO. Survival rate was the lowest among rats with severe ischemia, while none rat with mild ischemia died (Table 1).

Each group of rats was characterized by specific dynamics of BBF for 3 h after CAO. Periods of compensatory increase in BBF with subsequent restoration and increase in BBF were observed in rats with mild ischemia (Fig. 1, *a*). A compensatory BBF increase also occurred in animals with moderate ischemia, BBF being restored in 50% of them (Fig. 1, *b*). A wide variation range of the BBF value was characteristic of these animals. It was difficult to predict the dynamics of BBF on the basis of BBF decline within the first 15 min of CAO. In rats with severe ischemia BBF steadily decreased throughout the entire period of CAO. This led to death of 40%

TABLE 1. Effect of Semax on Local Blood Flow in Rat Brain Cortex Depending on the Severity of Brain Ischemia

Animals after CAO	*n*		BBF					Compensation of BBF		Mortality, %	
			initial		CAO, 15 min						
	number	%	ml/100 g× min	%	ml/100 g× min	%	*r*	CAO	CAO + semax	CAO	CAO + semax
All animals (BBF=30-97%)	43	100	35.01±2.52	100	19.45±1.56*	59.45±2.47	-0.54	±	+	22	14
Mild ischemia (BBF>70 %)	10	23	22.57±3.39°	100	17.95±2.06	81.51±2.85°°	-0.44	+	+	0	25
Moderate ischemia (BBF=50-70%)	20	47	36.24±3.14⁺	100	21.82±1.88*	60.16±1.35⁺⁺	-0.1	+	+	11	0
Severe ischemia (BBF<50%)	13	30	42.77±5.25⁺	100	16.94±1.83*	41.40±1.76°°	-0.62	—	+	60	33

Note. *n*) number of animals; presence (+) and absence (—) of compensatory increase in BBF; *r*) correlation coefficient; BBF (%) in comparison with the mean initial level taken as 100%. *$p<0.001$ compared with initial BBF; °$p<0.01$, °°$p<0.001$ compared with all animals; ⁺$p<0.01$, ⁺⁺$p<0.001$ compared with mild ischemia.

rats within 1 h of brain ischemia. The changes in BBF were directly proportional to the decrease in BBF within the first 15 min of CAO, being standard and predictable in rats from these group.

Semax rapidly restored and increased BBF over baseline level in rats with mild ischemia. However, as a result of wide variation range the differences between BBF during CAO before and after administration of semax were statistically insignificant ($p>0.05$, Fig. 1, *a*). The faster restoration of BBF under the action of semax was accompanied by death of 25% rats with mild ischemia. Semax had no effect on the dynamics of BBF in animals with moderate ischemia; however, it provided a high survival rate in this group (only 11% rats died). The preparation produced substantial effect on BBF in rats with severe ischemia (Fig. 1, *c*). The periods of compensatory increase in BBF, which were typical of rats with mild and moderate ischemia, were observed. The increase in BBF caused by semax in rats with severe ischemia was statistically insignificant ($p>0.05$) due to a wide variation range of BBF. However, qualitative changes were obvious: repeated compensatory increase in BBF was observed instead of its monotonous decrease, which reduced mortality to 25% (40% in the control).

Our results indicate that the peptide preparation semax produces equivocal effect in brain ischemia of various severity: it elicits positive effect on the BBF dynamics and survival rate in rats with severe brain ischemia, and negative effect in mild ischemia that can be spontaneously compensated. It is known that opioid peptides are effective in extreme states (stress and shock). It can be suggested that like opioid peptides, semax is highly effective in severe irreversible states, including brain ischemia. The effect of semax on survival rate in brain ischemia is similar to that of opioid peptides [8]. The ability of semax to compensate BBF disturbances in rats with decompensated brain ischemia agrees with the observation that semax and $ACTH_{1-24}$ elicit compensatory effect in rats with decompensated changes in central hemodynamics and liver blood flow but not in rats with compensated blood loss [4].

In contrast to enkephalins, which act as direct stimulators of lymph flow [8], semax had no effect on lymph flow via mesenteric LV. Presumably, the low lymph-stimulating activity of semax is associated primary with the absence of tyrosine, which provides lymph-stimulating activity of opioid peptides [9].

The different responses of BBF to the standard CAO are due to different realization of individual adaptive mechanisms to ischemia. A similar heterogeneity of individual reactions of animals to standard hypoxia, blood loss, and emotional stress has been shown by others [1,4,6]. The effective use of peptide bioregulators is possible only when individual reaction of the organism to damaging factor and severity of the disease are taken into consideration. This is confirmed by the findings that different brain structures have different peptide contents and that exogenous peptides produce different effects in animals with different resistance to emotional stress [6].

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Phospholipid Composition of Liver Mitochondria in Experimental Hemorrhagic Shock

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Heparin used as an anticoagulant in modeled hemorrhagic shock decreases the phosphatidylcholine and increases the phosphatidylethanolamine contents in the mitochondria. Accumulation of lysophosphatidylcholine in whole mitochondria and their inner membrane is observed in hemorrhagic shock. At the same time, hemorrhagic shock decreases phosphatidylcholine content in the inner and outer mitochondrial membranes and increases phosphatidylethanolamine content in the outer membranes. Modification of phospholipid composition of mitochondrial membranes is a mechanism responsible for impaired energy production in liver mitochondria in hemorrhagic shock.

Key Words: *phospholipids; mitochondria; liver, hemorrhagic shock*

Energy deficiency, which limits tissue functions, is a cause of shock irreversibility. Under normal conditions 95% of energy in the body is produced by the mitochondria [7]. In shock, energy production in liver mitochondria progressively impairs [9], and the mechanisms of this process remains unclear. There is evidence that alterations of phospholipid turnover in the mitochondria may reduce their functional activity [5,8,11]. It should be noted that in shock the liver is the first organ losing the energy-producing function [9].

In an attempt to elucidate the mechanisms of modification of energy production in the mitochondria and to find out the ways to correct these modifications, we decided to study changes in the phospholipid composition of liver mitochondrial membranes in experimental hemorrhagic shock. Since high doses of heparin, which is known to modify lipid metabolism, have been used to prevent blood coagulation in catheters, we examined the effect of heparin in the phospholipids of liver mitochondria.

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MATERIALS AND METHODS

Experiments were performed on 15 cats (body weight 3.0±0.5 kg) under Nembutal anesthesia (40 mg/kg intraperitoneally). Hemorrhagic shock was produced as described elsewhere [14]. For prevention of blood coagulation in catheters heparin was injected in a dose of 2000 U/kg. Blood was drained 30 min after the injection until blood pressure dropped to 40 mm Hg. Blood pressure was maintained at this level for 1 h. The cats were sacrificed after 1.5 h of blood loss. Intact animals injected and not injected with heparin served as controls. Material for the investigation was obtained 2 h after anesthesia. Liver mitochondria and their inner and outer membranes were

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