Effect of Semax and Semax-Heparin Mixture on Isolated Heart Activity after Total Ischemia in Rats

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Semax administered after 40-min total ischemia of isolated heart improved the recovery of heart rate and reduced myocardial contracture. When administered prior to ischemia, Semax exerted a negative effect on recovery of heart contractility. Semax-heparin mixture significantly improved the recovery of cardiac indices (end-diastolic pressure, heart rate, and relaxation rate) irrespective of administration schedule.

Key Words: *Semax; heparin; isolated heart; ischemia; reperfusion*

The peptide preparation Semax ($ACTH_{4-7}$-Pro-Gly-Pro) was developed in the early 1980s at the Laboratory of Regulatory Peptides, Institute of Molecular Genetics, Russian Academy of Science. Intense studies in the 1990s demonstrated high effectiveness of Semax in the treatment of pathology caused by impaired cerebral circulation and hypoxia [3,9] and in the postresuscitation recovery of physiological functions [2]. However, almost all the studies considered the effect of Semax on cerebral functions and there are no data on the effect of Semax on visceral activity and activity of isolated organs. Further on, little is know about its interaction with endogenous bioactive compounds. Of special interest is the combination of Semax with heparin, since this polyaminoglycan can form complexes with many peptides [7] including Semax [1]. This reaction can change the activity of both heparin and another element of the complex. In this connection, the aim of this study was to assess the effect of Semax alone and in combination with heparin on activity of isolated rat heart after total ischemia.

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MATERIALS AND METHODS

Experiments were carried out on Langendorff-Fallen-perfused isolated hearts [11] from outbred rats weighing 250-300 g. To produce ischemia, heart perfusion was stopped under normothermic conditions and resumed after 40 min. The recovery of cardiac activity was observed for 30 min after the onset of reperfusion. Contractile activity was measured under isovolumetric conditions by the method of Fallen. Developed pressure (contraction force), end-diastolic pressure (EDP), maximum rate of pressure rise and decay, and heart rate (HR) were recorded after 15-min perfusion before ischemia and after 5, 15, and 30 min of reperfusion. The data were expressed as percentage of the initial values.

The rats were divided into 7 groups (n=6-9): group 1 was control; groups 2, 4, and 6 animals received heparin (0.03 mg), Semax (0.03 mg), or Semax+heparin mixture (0.03+0.03 mg), respectively, immediately before ischemia; groups 3, 5, and 7 rats received heparin, Semax, and Semax+heparin, respectively, immediately after ischemia. The mixture of 0.1 mg Semax and 0.1 mg heparin (10 IU) dissolved in 1 ml distilled water was incubated at room temperature (25°C) for 1 h before use and infused into the aorta in a volume of 0.3 ml. We used Semax synthesized at

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the Russian Cardiology Research-and-Production Complex, Russian Ministry of Health and domestic high-molecular-weight heparin. The data were processed statistically using Student's t test and Fisher's exact probability test.

RESULTS

Preliminary study showed that Semax (0.03-0.3 mg), heparin (0.03 mg) and their 1:1 mixture caused no significant changes in the contractile activity of non-ischemic heart, but modulated it under pathological conditions. The effects of test drugs on myocardial contractility after total ischemia (percentage of the initial values) are presented in Table 1. The postischemic recovery of the heart function in the control group was accompanied by typical reperfusion injuries: considerable weakening of contractile activity and increasing contracture.

Pre- and postischemic administration of heparin had little effect on the recovery of heart function. Postischemic administration slightly increased EDP, which could be attributed to the formation of heparin-Ca^{2+} complex [6] preventing Ca^{2+} overload of cardiomyocytes during reperfusion. Heparin added during the first few minutes of reperfusion induced considerable, but transient increase in the contraction force against the background of very low HR.

The effects of Semax on the postischemic recovery of heart contractile activity depended on the administration schedule: preischemic administration significantly reduced contraction force ($p<0.05$) and heart rate ($p<0.05$), while postischemic administration considerably improved HR restoration ($p<0.05$ compared to the control) and reduced myocardial contracture ($p<0.05$). Significant changes in contraction force ($p<0.01$ compared to the control) were observed only for the first few minutes, which can be explained by a short-term effects of Semax on this parameter. It can be assumed that preischemic administration of Semax prevents the development of compensatory mechanisms through activation of cardiac metabolism with the corresponding increase in oxygen demand, which under conditions of oxygen deficiency deteriorate structural membrane integrity. Its cardiostimulating effect at the postischemic administration can be explained by improvement of oxygen supply [5]. This explanation is supported by the fact that postischemic administration of Semax predominantly modulated HR and relaxation rate, *i. e.* energy-dependent parameters.

Effects of pre- and postischemic administration of Semax-heparin mixture were similar to those of postischemic Semax alone. The mixture ranked slightly below Semax alone in therapeutic activity at postischemic administration, but induced a considerable

TABLE 1. Effect of Preischemic and Postischemic Administration of Semax, Heparin, and Semax-Heparin Mixture on Contractility of Isolated Rat Heart after Total Ischemia (% of initial values, $M\pm m$)

Index	Reperfusion, min	Group1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
EDP	30	893.3±110.4	853.3±174.9	306.7±43.4^{++}	1105.0±177.9°	440.0±40.0^{*+x}	304.0±77.5^{*+x}	200.0±17.8^{*+x}
HR	5	25.1±5.8	44.0±5.7	32.1±5.5	27.1±8.6	55.5±8.5^{*x}	41.8±15.9	49.1±5.5^{*x}
	15	39.9±9.3	48.2±12.6	63.3±10.9	50.1±12.3	82.2±11.4^{*x}	89.2±6.6^{*+x}	102.3±16.9^{*x}
	30	75.7±10.4	56.3±11.3	72.3±3.2	59.0±11.3	101.8±10.4^{*ox}	99.4±9.5^{*+ox}	102.3±10.58^{*+x}
Contraction force	5	69.3±11.8	69.5±10.2	133.9±23.7*	61.8±10.1°	88.7±2.7^{*x}	103.0±9.6^{*+x}	118.7±17.2^{*+x}
	15	83.0±14.5	71.6±12.7	82.6±49.3	49.7±7.5°	89.3±3.98^{x}	84.4±5.4^{x}	93.4±7.56^{x}
	30	66.8±12.7	86.5±13.2	88.3±5.2	44.3±3.7$^{*\circ}$	85.0±5.3^{x}	74.8±8.65^{x}	92.5±10.2^{x}
Contraction rate	5	51.0±13.5	40.5±8.7	68.3±19.7	40.0±12.1	69.8±9.0^{+}	85.0±9.0^{*x}	69.5±9.6
	15	57.1±10.0	44.0±4.7	74.2±12.0	36.6±3.3°	99±5.9^{*+x}	83.2±8.7^{*x}	80.2±7.5^{*x}
	30	71.7±12.0	39.7±6.6	95.0±15.2	35.0±3.1$^{*\circ}$	93.8±6.5^{*x}	86.8±9.2^{*x}	77.3±11.3^{*x}
Relaxation rate	5	34.6±4.1	40.2±5.7	46.0±9.8	25.5±1.8^{+}	55.0±9.8^{x}	55.0±13.4^{x}	66.0±14.4^{x}
	15	50.1±6.1	48.5±5.0	59.7±10.3	30.8±3.5$^{*\circ}$	79.6±5.6^{*+x}	74.4±10.7^{*+x}	88.0±9.39^{*+ox}
	30	52.4±8.17	44.7±4.6	63.7±8.9	30.4±4.0$^{*\circ}$	81.7±5.8^{*+x}	77.2±6.32^{*+x}	85.8±12.6^{*+x}

Note. Significant differences: *compared to the control (group 1); +compared to group 2; °compared to group 3; xcompared to group 4.

increase in contraction force during the first few minutes of reperfusion ($p<0.05$ compared to the control) typical of heparin. Unlike its components, the mixture showed considerable adaptogenic properties after preliminary administration improving myocardial resistance to ischemic damage. EDP, HR, and relaxation rate differed significantly not only from the controls ($p<0.05$), but also from respective values after preischemic administration of Semax ($p<0.01$) and heparin ($p<0.05$) alone.

The mechanisms of action of Semax-heparin mixture remain unclear. Antiischemic properties of heparin are well known [10]; however, they are observed after its systemic administration. Since heparin easily forms complexes with bioactive plasma components, it can be suggested that the observed antiischemic effect is due to these compounds rather than heparin itself. This suggestion is supported by the fact that heparin complexes are superior to heparin alone in antihypoxic activity [4]. Depending on the experimental conditions, heparin either increases oxygen consumption and activates oxidative phosphorylation in mitochondria or inhibits respiratory enzymes and activates glycolysis [8]. It can be assumed that at the preliminary administration of the mixture, heparin protects Semax from proteolysis, thus reducing its metabolic effects, but promotes their manifestation in the postischemic period.

Thus, Semax shows no significant effects on isolated rat heart under normal conditions, but significantly modulates postischemic recovery of myocardial contractility. The character of this modulation depends on the administration schedule. Heparin potentiates the antihypoxic effects of Semax.

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