DOI 10.1007/s10517-017-3862-3

Changes in Sympathetic Innervation of the Heart in Rats with Experimental Myocardial Infarction. Effect of Semax

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 163, No. 5, pp. 570-573, May, 2017
Original article submitted October 7, 2016

The effect of peptide Semax on remodeling of cardiac sympathetic innervation was examined in rats with experimental myocardial infarction. In 28 days after ischemia/reperfusion injury, Semax diminished the growth of sympathetic innervation of ventricular septum, although it produced no effect on the density of β_1 and β_2 adrenoceptors.

Key Words: *myocardial infarction; Semax; autonomic nervous system*

Hyperactivation of the sympathetic nervous system plays a deleterious role during the postinfarction period and promotes heart failure [5]. Activation of the sympathetic nervous system after myocardial infarction is aimed at compensation of hemodynamic abnormalities and normalization of both BP and stroke volume. However, these parameters of cardiovascular system cannot be fully restored due to partial cardiomyocyte death. In this relation, hyperactivity of sympathetic nervous system remains important also in the late period after myocardial infarction by augmenting the cardiac pre- and afterload, which unfavorably affects the development of contractile dysfunction of the myocardium [5].

It is important that remodeling of sympathetic innervation is an essential part of general myocardial rehabilitation, which implicates not only the heart, but the vascular bed as well. During this process, elevation in blood catecholamines is accompanied with the changes in sympathetic innervation density of the heart and in the density of adrenoceptors [5,6]. Semax ($ACTH_{4-7}$+Pro-Gly-Pro fragment) is a neuroactive peptide, which can affect remodeling of autonomic innervation [1].

This work was designed to study the effect of Semax on the density of sympathetic innervation and adrenoceptors in the ventricular septum of the rat heart after experimental myocardial infarction.

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MATERIALS AND METHODS

Experiments were carried out on outbred male albino rats (n=60) weighing 300-400 g. All procedures with animals were carried out in strict adherence to Order No. 755 of the Ministry of Health of USSR (August 12, 1977) and approved by the Biomedical Ethics Committee of M. V. Lomonosov Moscow State University. The animals were maintained under standard vivarium conditions with controlled day-night cycle and free access to the standard mixed fodder for rodents and water. The rats were randomized into 5 groups of 12 animals each: intact control (IC group), irreversible ischemia (II group), II+Semax group, ischemia/reperfusion (IR group), and IR+Semax group.

Ischemia and ischemia/reperfusion were modeled under deep anesthesia with intraperitoneal chloral hydrate (400 mg/kg) according to originally modified method of Selye (without rib excision and without artificial ventilation) [7]. The left coronary artery was ligated below the left atrial appendage, so 30% left ventricle volume was in the risk area. Reperfusion was performed in 2.5 h after ligation. This period corresponded to the reperfusion schedule used in clinics during myocardial infarction, and it made possible to observe the development of reperfusion syndrome.

Semax (150 μg/kg) was injected intraperitoneally in 15 min and in 2 h 15 min after surgery (*i.e.*, the second injection was made 15 min prior to reperfusion) and then daily during the following 6 days. The rats in II and IR groups were injected with equivalent amount of physiological saline.

For histological examination, the hearts were excised from the rats deeply narcotized with chloral hydrate and decapitated.

In 3 days after infarction, the volume of damaged cardiac tissue was assessed in 1-1.5 mm sections stained with 2,3,5-triphenyltetrazolium chloride. Staining was arrested with 10% formalin, thereupon the sections were placed between the slides and scanned from both sides. AUC software was employed to assess the size of necrosis area as the percentage of damaged area of the total area of left ventricular sections. On postsurgery day 28, the volume of damaged tissue was determined as the ratio of scar tissue weight to total weight of the left ventricle.

In 28 days after surgery, sympathetic innervation density in the ventricular septum (*i.e.*, in conventionally intact myocardium) was determined with a 30-min incubation of the specimens in 0.1 M PBS (pH 7.2) supplemented with 2% glyoxylic acid and 10% sucrose. Then they were photographed under an Axiovert 200 luminescent microscope at 440-480 nm using an AxioCamHiRes digital camera equipped with ×40 objective. Paint.NET 3.5.8 software was employed to superimpose a grid across the images and to count the number of grid intersections crossed by stained sympathetic nerve terminals per 100 grid intersections.

On postsurgery day 28, the density of β_1 and β_2 adrenoceptors was assessed immunohistochemically in 5-μ paraffin sections according to the Abcam protocol. The sections were stained with primary rabbit polyclonal antibodies against rat adrenoceptors and secondary goat antibodies against rabbit immunoglobulins labeled with horseradish peroxidase. The relative area of stained sites reflecting the density of adrenoceptors was evaluated using Image-ProPlus software.

The results were analyzed statistically using Statistica 6.0 software. The data on damage area are summarized as m±95%CI, and the densities are presented as median (Me) with the interquartile range (IQR=Q_1-Q_3). Significance was assessed with Mann—Whitney test at $p<0.05$.

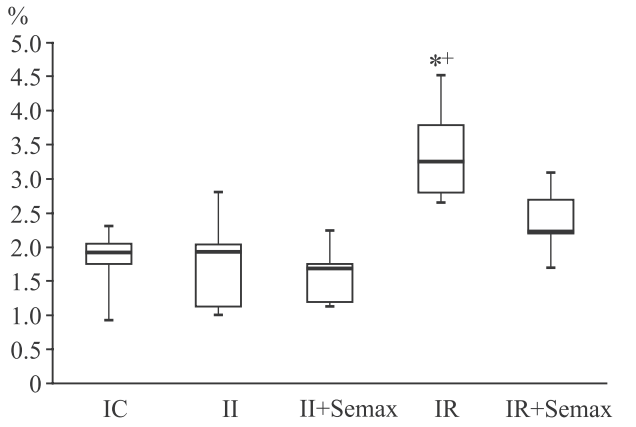

Fig. 1. Effect of Semax on the density of sympathetic innervation in the myocardium in II and IR groups. $p<0.05$ in comparison with *IC, +(IR+Semax).

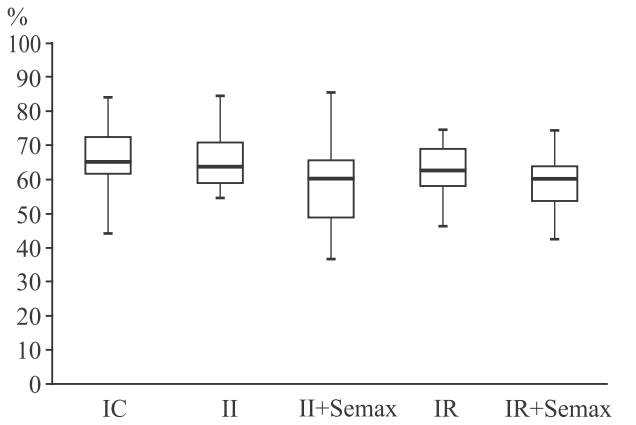

Fig. 2. Effect of Semax on the density of β_1 adrenoceptors in ventricular septum of II and IR group rats.

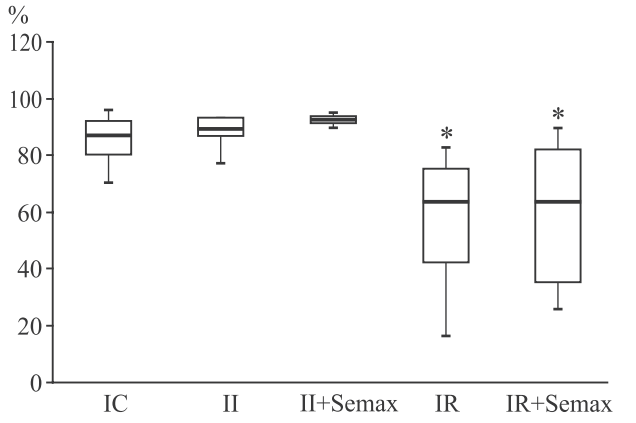

Fig. 3. Effect of Semax on the density of β_2 adrenoceptors in ventricular septum of II and IR group rats. *$p<0.05$ in comparison with IC group.

RESULTS

On postsurgery day 3, the damaged area in IR group was significantly smaller than in II group (12.7±4.4 and 18.0±3.5%, respectively). In both groups, Semax produced no effect on the size of postinfarct necrosis region. On postsurgery day 28, the sizes of scars in II and IR groups were similar (17.27±4.4 and 18.4±3.5%, respectively).

In the IC group, the density of sympathetic innervation in conventionally intact region of the myocardium (ventricular septum) was 1.94 (IQR=0.27). In

II group, this density was insignificantly greater (2.37, IQR=0.9), whereas in II+Semax group it was insignificantly smaller (1.99, IQR=0.55) than the control value. In contrast, the density of sympathetic innervation in IR group significantly surpassed the control value and attained 3.34 (IQR=0.99). Semax reduced the density of innervation in IR group to 2.02 (IQR=0.47; Fig. 1).

In IC group, density of β_1 adrenoceptors was 66 (IQR=11). In both II and IR groups, it was virtually identical: 66 (IQR=12) and 61 (IQR=11), respectively. The Semax-treated groups demonstrated practically identical density of β_1 adrenoceptors: in II+Semax and in IR+Semax groups, the corresponding values were 58 with IQR=17 and 67 with IQR=10 (Fig. 2). In IC and II groups, the densities of β_2 adrenoceptors did not differ significantly: 86 (IQR=6) and 89 (IQR=12), respectively. In contrast, this density was smaller in IR group (56 with IQR=33). Semax produced no effect on the density of β_2 adrenoceptors in rats with II (in II+Semax group), which was 93 (IQR=2) and did not restore it during IR (in IR+Semax group), which was 60 (IQR=47; Fig. 3).

The large-scale death of cardiomyocytes during postinfarction period results in systolic dysfunction of the myocardium, drop of cardiac output, decrease in BP, and rearrangement of sympathetic branch of autonomic nervous system [5]. To compensate for these alterations, the baroreflex system and the renin—angiotensin—aldosterone axis become activated, which augments sympathetic influences and elevates blood norepinephrine [9]. At this, the myocardium infarction is compensated only partially, so up-regulation of sympathetic system is maintained for a long period [5].

The development of inflammation in ischemic area elevates production of growth factors resulting in compensatory hypertrophy of the myocardium, vascularization of the penumbra region and the spread of sympathetic terminals prompted by growth factors [3,4,9]. It is interesting, that no significant increase in the density of the septal sympathetic terminals was observed in II group in contrast to IR group. Thus, reperfusion accelerates the remodeling of sympathetic innervation in conventionally intact zone, because a large number of inflammatory cells, which produce the growth factors [8], invade it. At this, the negative feedback down-regulates expression of membrane adrenoceptors thereby diminishing sensitivity of the tissue to catecholamines. In this study, we observed this process for β_2 adrenoceptors.

According to our studies, Semax moderates the hyperactivity of sympathetic nervous resulted from myocardial infarction. Actually, it diminishes HR, the end-diastolic pressure, and apoptotic rate of the cardiomyocytes resulting in a decrease of sympathetic innervation of the heart [1]. These phenomena can be related to the anti-inflammatory action of Semax, which down-regulates the secretion of growth factors [2] and therefore diminishes the density of sympathetic innervation. As a result, Semax abates the hyperactivity of sympathetic system. Since the inflammatory reaction is especially pronounced during reperfusion, the effect of Semax is also stronger under these conditions.

The study was supported by the Russian Foundation for Basic Research within a framework of research project No. 16-34-00902mol_a.

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