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Changes in Sympathetic Innervation of Rat Caudal Artery in Experimental Myocardial Infarction. Effect of Semax Peptide

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Activation of the sympathetic nervous system aggravates the course of myocardial infarction. Semax peptide moderated the degree of this activation and prevented the increase in the density of sympathetic endings in rat caudal artery in 28 days after ischemia or ischemia/reperfusion. The peptide reduced the density of α-adrenoreceptors in the caudal artery of rats with myocardial infarction. Semax produced no effect on β-adrenoreceptors in both experimental models. The experiments on isolated segments of the caudal artery revealed reduced vascular responsiveness to electrical stimulation and norepinephrine infusion in rats treated with Semax after ischemia/reperfusion injury.

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

Activation of the sympathetic nervous system (SNS) during the development of myocardial infarction is aimed at compensation of reduced cardiac output and BP. Myocardial infarction leads to necrosis of the myocardial of that does not completely recover, while enhanced activity of SNS persists for a long time thereby increasing the load to the heart. Elevation of extracellular norepinephrine (NE) affects the density, subtype composition, and/or sensitivity adrenoreceptors (AR), as well as the density of sympathetic innervation in the heart.

Apart from the myocardium, other organs and tissues are exposed to redundant catecholamine signaling. We have previously demonstrated that BP rise provoked by intravenous infusion of α-AR agonist phenylephrine in rats after experimental myocardial infarction modeling was less pronounced than in controls [1], probably because of not only heart failure, but also changes in the vascular bed [1]. In these studies, Semax (ACTH4-7Pro-Gly-Pro fragment) reduced SNS hyperactivation and postinfarction shifts of BP response to phenylephrine. SNS hyperactivation is an important clinical problem, because this redundant activity provokes numerous complications of myocardial infarction, including ventricular tachycardia.

This work was designed to study postinfarction remodeling of innervation in the vascular bed of rat caudal artery at the molecular, morphological, and physiological levels under control conditions and after treatment with Semax.

MATERIALS AND METHODS

Experiments were carried out on random-bred male albino rats weighing 300-400 g. The animals were maintained under vivarium conditions with controlled day-night cycle and received water and pelleted food *ad libitum*. Tissue specimens were isolated under intraperitoneal chloral hydrate narcosis (400 mg/kg). Irreversible ischemia (IrI) was modeled according to Selye by occlusion of the left coronary artery at a distance of 3-4 mm in apical direction from the left atrial appendage. This procedure produces the ischemic region involving 30% of the left ventricle (apex and anterior wall). This routine model of ischemia is used in many studies yielding reliable and reproducible results [1]. In the ischemia/reperfusion (I/R) model, ischemia was followed by reperfusion in 2.5 h after occlusion of

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the left coronary artery. The development of myocardial infarction was verified on day 28 after surgery: the heart was isolated, the left ventricle was weighed, then the scar tissue was isolated and weighed separately to calculate the percentage in the left ventricle occupied by the scar. Semax was injected intraperitoneally in 15 min and in 2 h 15 min after surgery, thereupon it was injected daily for 6 days in a dose of 150 μg/kg. The controls (n=8) were similarly injected with physiological saline. The following experimental groups were formed: IrI, IrI+Semax, I/R, and I/R+Semax (each group included 8 rats). Segments of the caudal artery were taken on day 28 after surgery (end of remodeling processes).

To visualize the sympathetic endings, the vessels were incubated for 30 min in 0.1 M PBS (pH 7.2) containing 2% glyoxylic acid and 10% sucrose and photographed under an Axiovert 200 microscope (λ=440-480 nm) equipped with AxioCamHiRes digital camera (objective ×40, Fig. 1, *a*). In Paint.NET 3.5.8 utility, a grid was applied on the image and grid intersections with sympathetic nerves in 20-30 randomly oriented images per specimen were counted. The data are presented as the number of crossings per 100 grid knots referred as relative innervation density (RID, Fig. 1, *b*).

The densities of α_1-, β_1-, and β_2-AR were determined immunohistochemically in 5-μ paraffin sections according to Abcam antibody producer guide using primary rabbit polyclonal antibodies to rat IgG and secondary horseradish peroxidase labeled goat polyclonal antibodies to rabbit IgG. An Image-ProPlus software utility was used to assess the relative stained area that reflected AR density (Fig. 1, *c*, *d*).

Physiological experiments were carried on 10-mm caudal artery segments. The arterial segment (second from the tail base) was isolated and the endothelium was removed. The proximal and distal ends of the segment were pulled on the bare end of insulated metal cannula and a polyethylene catheter with internal diameter of 0.5 mm, respectively. Then the mounted segment was placed into experimental chamber filled with Krebs–Henseleit solution containing (in mM): 119 NaCl, 25 KCl, 4.7 KH_2PO_4, 1.18 $MgSO_4 \times 7H_2O$, 1.17 $NaHCO_3$, 5.5 Gluc×H_2O, 1.5 $CaCl_2 \times 2H_2O$ at 37°C (pH 7.35). The vascular segment was stretched out to its *in situ* length; thereupon the cannula and catheter were fixed. The segment was perfused with internal and external flow rates of 2 and 7 ml/min and perfusion pressure being recorded. To assess vasoconstriction of the caudal artery in response to the release of endogenous NE, we used transmural electrical stimulation of efferent nerve endings via the cannula and external gold electrode. Stimulation was performed with 200-mA rectangular pulses of alternating polarity. The

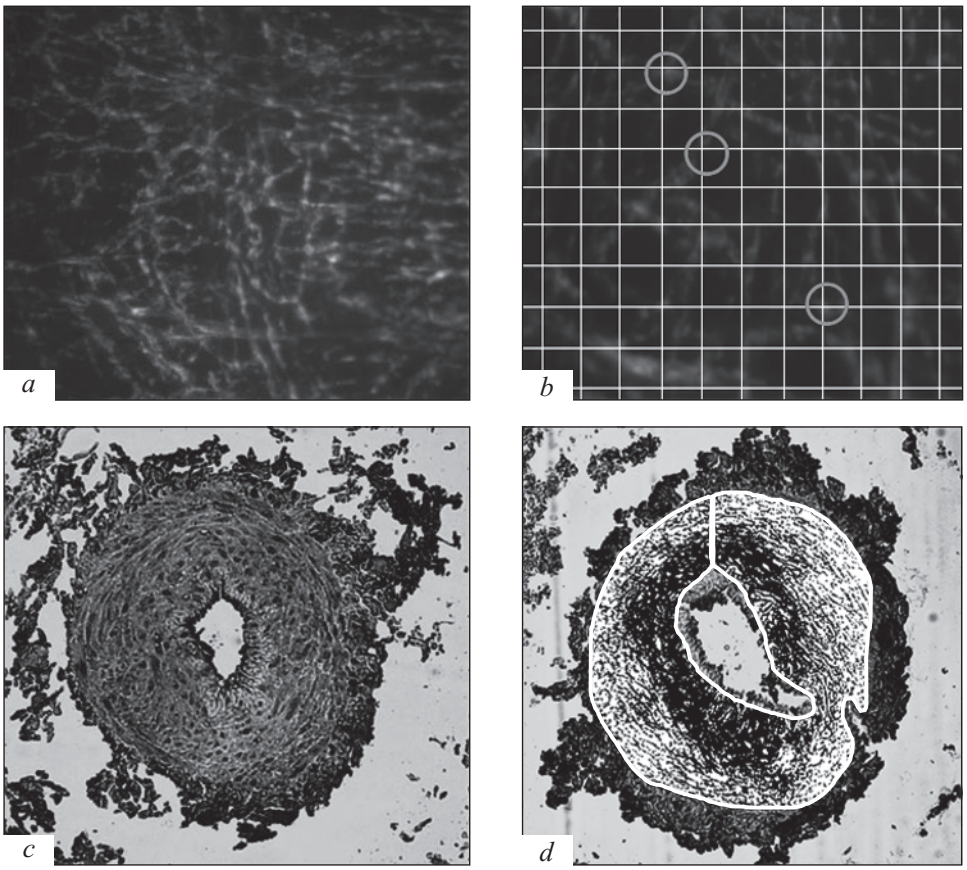

Fig. 1. Methods used to assess sympathetic innervation (*a*, *b*) and AR (*c*, *d*) densities in rat caudal artery. *a*) Artery stained with glyoxylic acid, ×40; *b*) count of sympathetic innervation density according to the number of crossings of fluorescent sites with intersections of superimposed grid; *c*) cross-section of the artery stained with horseradish peroxidase; *d*) cross-section of the artery processed with Image-ProPlus software. The white line shows the region where AR density was counted.

frequency of stimulating current and the total duration of pulse train were as follows: 1 Hz, 1500 msec; 3 Hz, 500 msec; 5 Hz, 300 msec; 10 Hz, 150 msec; 20 Hz, 75 msec; 30 Hz, 50 msec; 40 Hz, 37.5 msec; 50 Hz, 30 msec; 60 Hz, 25.5 msec; 70 Hz, 21 msec; and 80 Hz, 19 msec. These parameters ensured stimulation of the nerves, but not vascular smooth muscles [4,5].

To examine the response of the caudal artery to AR agonist, the vascular segment was perfused with NE solution in the accumulating mode at concentrations from 3×10^{-8} to 10^{-5} M. To this end, the vascular segment was mounted and fixed in the chamber, adapted under resting conditions for 30 min, stimulated electrically as described above, washed with physiological saline for 15 min, and perfused with NE solution. At the end of experiment, the absence of the endothelium was verified by combined perfusion with NE and 10^{-6} M acetylcholine. The vessels demonstrating significant endothelium-dependent relaxation were excluded from the analysis.

The data were analyzed statistically using Statistica 6.0 (StatSoft) software and nonparametric Mann–Whitney test at $p<0.05$. The results are summarized as *m*±*SEM*.

RESULTS

IrI and I/R in the basin of the left coronary artery resulted in the formation of a myocardial scar occupying 17±2 and 18±2% of the left ventricular mass (relative scar mass, RSM), respectively. Semax produced no effect on the size of lesion: in IrI+Semax and I/R+Semax groups, RSM values were 18±3 and 18±6%, respectively.

In healthy rats, RID in the caudal artery was 22±9 arb. units. Experimental myocardial infarction significantly increased RID by 1.5 and 1.3 times in IrI and I/R models, respectively (Fig. 2, *a*). Semax significantly prevented the increase in RID after IrI: this parameter remained at the control level (23±13 arb. units), but had no effect on RID after I/R (Fig. 2, *a*).

The relative density of α_1-AR did not differ in the control and experimental groups (Fig. 2, *b*). The evident drop of this density in I/R group by 20% was insignificant (p=0.17). In contrast, Semax significantly elevated the density of α_1-AR relative in rats with IrI and I/R up to 56±29 and 41±19%, respectively (Fig. 2, *b*). It is noteworthy that two models demonstrated

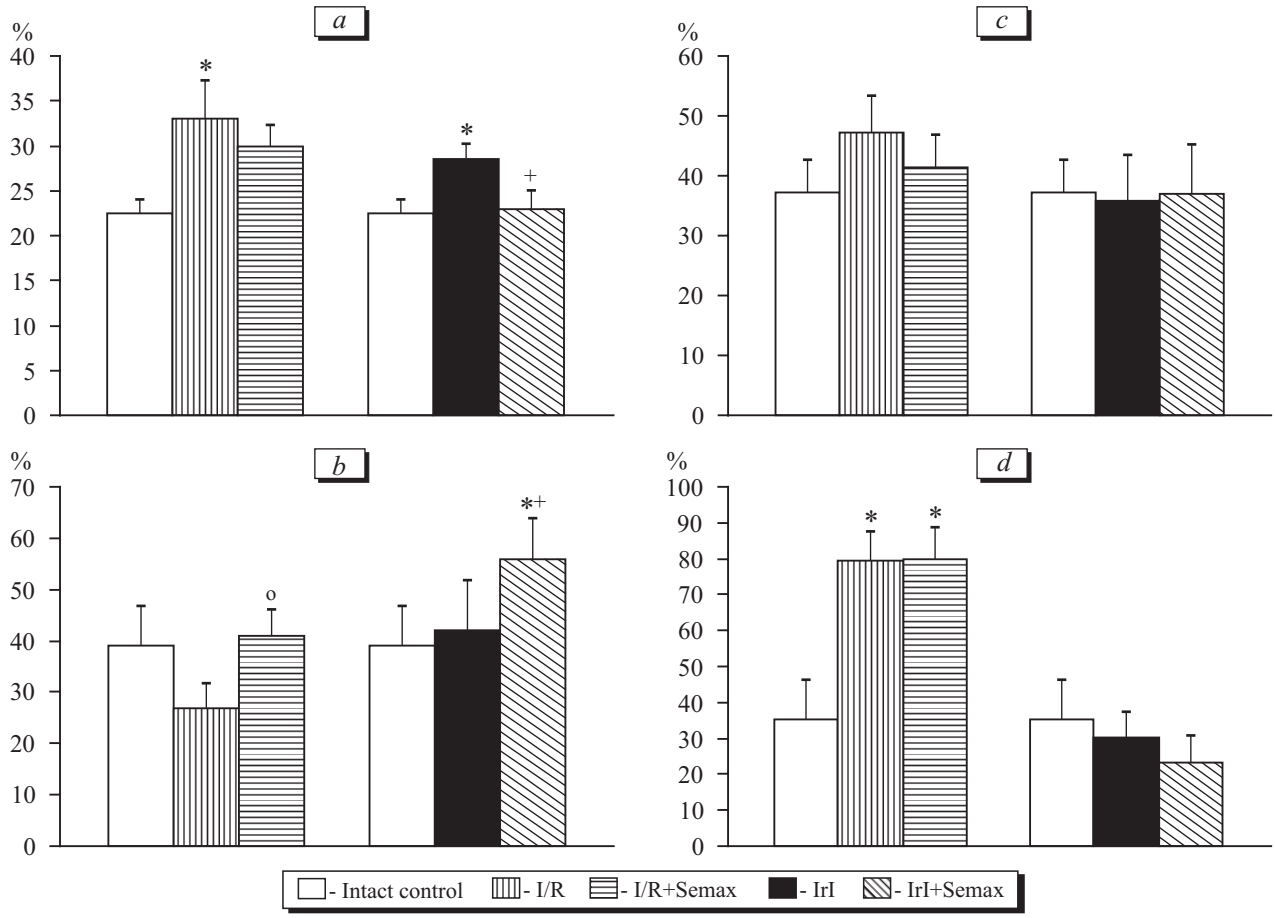

Fig. 2. Effects of IrI, I/R, and Semax on the density of sympathetic endings(*a*), α_1-AR(*b*), β_2-AR (*c*), β_1-AR (*d*). $p<0.05$ in comparison with *intact control, ^{0}I/R, $^+$IrI.

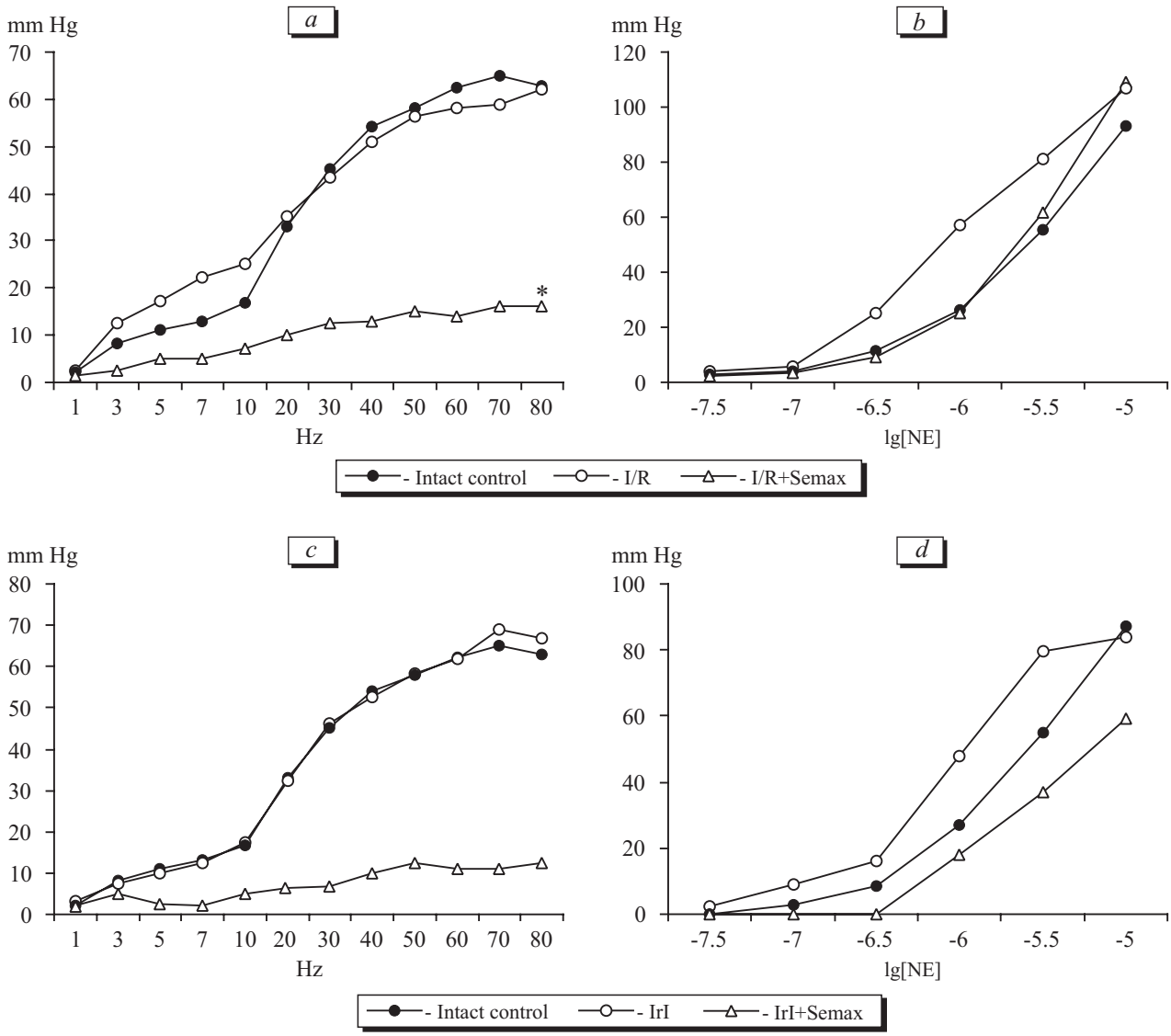

Fig. 3. Effect of transmural electrical stimulation of sympathetic endings (*a*, *c*) and perfusion with increasing NE concentrations (*b*, *d*) on perfusion pressure applied to isolated segment of rat caudal artery isolated from the rats with I/R (*a*, *b*) and IrI (*c*, *d*) models of myocardial infarction.

different changes in the relative density of β_1-AR. IrI produced no effect on this parameter, but I/R increased it by 2.6 times (Fig. 2, *d*). Semax produced no effect on the relative density of β_1-AR in both models (Fig. 2, *d*). Both models of myocardial infarction produced no effect on the relative density of β_2-AR (Fig. 2, *c*). Semax exerted no effect on this value in both models (Fig. 2, *c*).

Transmural electrical stimulation resulted in the release of NE from sympathetic endings in vascular wall [4], dose-dependent vasoconstriction, and elevation of perfusion pressure (Fig. 3, *a*). I/R produced no effect on arterial response to stimulation of nerve endings. The peak of stimulation-induced vasomotor reaction was observed at 80 Hz. In rats with I/R, Semax significantly decreased the peak of vasomotor reaction by 3 times (attained at 50 Hz) in comparison with I/R group (Fig. 3, *b*).

IrI alone or in combination with Semax produced no effect on vasoconstrictor response induced by electrical stimulation (Fig. 3, *c*).

Perfusion of isolated caudal artery with increasing concentrations of NE induced similar dose-dependent response in all examined groups (Fig. 3, *b*, *d*). Thus, Semax did not modulate NE-induced vasoconstriction in both models of myocardial infarction.

It is a common knowledge that myocardial infarction stimulates SNS in a stage-dependent way. At first, firing in sympathetic nerves increases together with up-regulation of catecholamine synthesis. Then, the number and sensitivity of receptors as well as concentration of neurotransmitters also change.

In the models of myocardial infarction, the researchers usually focus on remodeling of the coronary basin and alterations in cardiac innervation, while the structural and functional changes in the vessels of other organs receive no proper attention [2,3].

It is of importance that in our studies, I/R and IrI models induced the growth of sympathetic endings in caudal artery, which we previously observed in the myocardium (unpublished data). When describing this cardiac phenomenon, the researchers focus on extra production of inflammatory cytokines and growth factors in the ischemic penumbra. Augmented innervation of the caudal artery can result from production of these growth factors as well as from activation of SNS and renin–angiotensin–aldosterone system, whose components can stimulate the growth processes [6-9]. We have previously demonstrated reduced pressor reaction to intravenous infusion of α-adrenomimetic in rats with myocardial infarction; this phenomenon was related to peculiarities of the vascular bed and not to the development of cardiac failure [1].

In the present study, Semax reduced the density of sympathetic endings in the caudal arterial wall and increased the density of α-AR in arterial smooth muscle cells in the delayed postinfarction period. The data obtained on isolated segments of caudal artery differ from those yielded previously by *in vivo* experiments [1]. Here, Semax reduced the response to stimulation of α-AR partially due to inhibition of sprouting of the novel nerve endings, which compensatory up-regulated the sensitivity of the postsynaptic membrane. At the same time, Semax decreased sensitivity of the caudal artery to electrical stimulation probably due to a decrease in the density of sympathetic nerve endings and/or diminution of NA content in presynaptic vesicles.

The changes in density of β_1-AR in I/R model were not accompanied with alterations of other innervation parameters of the caudal artery. Probably, in this vascular region, the major role is played by the α_1-adrenergic effect of SNS on the vessels, whereas the presynaptic β_2-AR are not involved in remodeling.

It is an established fact that chronic sympathetic denervation and a long-term drop of the transmural pressure increase the sensitivity of resistance vessels to the constrictor agents [4]. Interestingly, the density and affinity of arterial α-AR did not change in these experiments [4]. In present study, sensitivity of the caudal artery to NE on postinfarction day 28 increased, and this phenomenon was accompanied with elevation in the density of α-AR. Probably, these peculiarities are somewhat related to specificity of the caudal artery known to be involved in thermal control in the organism.

Thus, we have established that the delayed period of experimental myocardial infarction is characterized by enhancement of sympathetic innervation not only in the heart, but also in the caudal artery. Peptide Semax exerted the corrective effect on this process.

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