

INCORPORATION OF S^{35} -METHIONINE INTO PROTEINS OF HEART MUSCLE AND CERTAIN ORGANS IN EXPERIMENTAL NEUROGENIC-AUTOALLERGIC MYOCARDIAL INFARCTION

I. P. Gerelyuk

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During stimulation of the vagus nerve in rabbits for 25-60 days an increase in the γ -globulin and decrease in the albumin fraction, together with an increase in the intensity of synthesis of serum proteins in organs associated with immunogenesis (spleen, lymph glands) were found. Anticardial autoantibodies appeared in the blood. Incorporation of radiomethionine into heart muscle proteins was increased toward the 60th day of vagal stimulation, i.e., in the period of development of microinfarcts, and also in the zone of the infarct arising under the influence of myocardiocytotoxic serum.

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The possibility of formation of anticardial autoantibodies has been demonstrated both clinically [6, 9] and experimentally [7]. The importance of autoallergy in the pathogenesis of myocardial infarction has been confirmed by the new experimental model of neurogenic-autoallergic myocardial infarction developed by V. T. Antonenko [1].

The object of the present investigation was to continue the study of the role of the immunologic component in the pathogenesis of infarction and also to study some aspects of protein metabolism in the myocardium during development of the pathological changes.

EXPERIMENTAL METHOD

Experiments were carried out on 53 rabbits. To produce neurogenic-autoallergic myocardial infarction the common trunk of the left vagus nerve was stimulated for a long period (25-60 days) by a metal ring applied at the level of the 4th cervical vertebra (Antonenko's method). The animals of one group (stimulated for 25 days) received an injection of myocardiocytotoxic serum (2 ml/kg body weight in a titer of 1:320) obtained by immunization of hens with antigen from a degenerating heart. The total serum proteins were investigated refractometrically and the protein fractions by electrophoresis on paper. Protein synthesis was estimated from the intensity of incorporation of S^{35} -methionine. Data for the utilization of free labeled amino acids in the synthesis of serum proteins of organs associated with immunogenesis (spleen, lymph glands) were taken into consideration [4]. Labeled methionine was injected in a dose of 20,000 pulses/min/g body weight. The animals were sacrificed after 18 h by air embolism. Proteins were isolated by precipitation with trichloroacetic acid and were defatted to constant weight with alcohol and ether.

TABLE 1. Serum Protein Fractions and Total Serum Protein of Rabbits during Prolonged Stimulation of Left Vagus Nerve, $M \pm m$

Procedure	Total protein	Albumins	Globulins			Albu-min-glo-bulin ratio
			α	β	γ	
Control	7.2 ± 0.09	61.9 ± 0.68	13.2 ± 0.42	11.6 ± 0.28	13.3 ± 0.36	1.6 ± 0.03
Stimulation of vagus nerve (25 days)	7.1 ± 0.08 $P > 0.2$	57.8 ± 0.65 $P < 0.001$	13.8 ± 0.26 $P > 0.05$	10.9 ± 0.28 $P = 0.1$	17.5 ± 0.4 $P < 0.001$	1.3 ± 0.03 $P < 0.001$

Department of Pathological Physiology, Ivano-Frankovsk Medical Institute (Presented by Active Member of the Academy of Medical Sciences of the USSR S. E. Severin). Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 66, No. 8, pp. 23-26, August, 1968. Original article submitted July 20, 1966.

TABLE 2. Intensity of Incorporation of S^{35} -Methionine into Proteins during Prolonged Stimulation of Vagus Nerve in Rabbits (in pulses/min/2 mg protein), $M \pm m$

Procedure	Radioactivity						
	blood serum			spleen	adren- alin	lymph glands	
	total proteins	albumins	glo- bulins			retroperi- toneal	mesen- teric
Control	125 \pm 5	78 \pm 7	131 \pm 7	142 \pm 8	151 \pm 4	159 \pm 11	135 \pm 8
Stimulation of nerve (25 days)	148 \pm 6 $P=0,05$	82 \pm 10 $P>0,05$	162 \pm 4 $P<0,05$	202 \pm 8 $P<0,001$	190 \pm 2 $P<0,05$	211 \pm 7 $P=0,001$	174 \pm 12 $P=0,05$

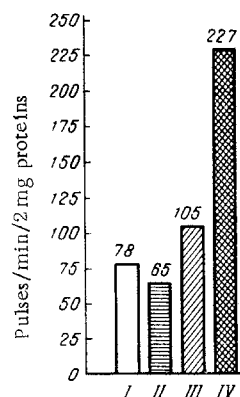


Fig. 1. Incorporation of S^{35} -methionine into rabbit heart muscle proteins (pulses/min/2 mg protein). I) Control; II) stimulation of vagus nerve for 25 days; III) ditto for 60 days; IV) injection of myocardiocytotoxic serum.

The radioactivity was determined in samples weighing 2 mg by an end-type counter in conjunction with a B-2 apparatus. Anticardial autoantibodies were determined by Boyden's indirect hemagglutination reaction and by the complement fixation reaction.

EXPERIMENTAL RESULTS

On the 25th day of stimulation of the vagus nerve the content of the serum albumin fraction in the rabbits' blood showed a statistically significant decrease, while the γ -globulin level, on the other hand, was raised; no significant change was found in the α - and β -globulin fractions or in the total serum protein (Table 1).

By the 25th day of stimulation of the nerve a deep Q wave in conjunction with a negative T wave was observed on the ECG, and the appearance of foci of infiltration of round cells, with stasis in the small veins and considerable loosening of the structure of the stroma were detected histologically. Anticardial antibodies were found in 9 of the 15 rabbits in titers of between 1:40 and 1:80.

Incorporation of S^{35} -methionine into the proteins of the blood serum, spleen, lymph glands, and adrenals was next studied. Consideration was paid to data in the literature [8] indicating that nonspecific globulins are synthesized by lymphatic cells of the same type as those which synthesize antibodies (Table 2).

As Table 2 shows, a statistically significant increase in the intensity of S^{35} -methionine incorporation was observed into total serum proteins (18.3%), globulins (28.2%), and also into proteins of the spleen (42%), lymph glands (33%), and adrenals (25%).

The increase in incorporation of the amino acid into globulins, accompanied by a simultaneous increase in the γ -globulin fraction, thus indicates increased formation of γ -globulins in the body.

By the 60th day of vagal stimulation, just as in Antonenko's experiments, microinfarcts were found histologically in the left ventricle. Characteristically the autoantibody titer by this time had risen from 1:40 to 1:120. Injection of myocardiocytotoxic serum against the background of vagal stimulation caused the development of a massive myocardial infarct with characteristic electrocardiographic and histological changes.

Incorporation of S^{35} -methionine into proteins of the heart muscle affected by degenerative changes and during development of infarcts in the organs is shown in Fig. 1. Increased incorporation of radio-methionine into the myocardial proteins on the 60th day of vagal stimulation and also in the zone of the infarct after injection of myocardiocytotoxic serum may be regarded as having a common genesis, presumably associated with changes in properties of the heart muscle proteins. As Pasynskii [2, 3] has shown, a number of procedures leading to structural or aggregative changes in proteins bring about an increase in the sorption properties of the proteins toward amino acids, especially S^{35} -methionine. Such changes in the state of aggregation of proteins may also be produced by formation of an antigen-antibody complex [5].

These facts suggest that anticardial autoantibodies appearing during chronic vagal stimulation are not "silent witnesses" but, by combining with antigens of the degeneratively changed myocardium, they may intensify and extend the neurogenic degenerative lesion in it.

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