Analysis of the data obtained by estimation of the size of SV leads to the conclusion that they constitute a heterogeneous population and that each different functional state of the neuromuscular synapse corresponds to its own type of distribution of SV by this parameter. This is evidence that heterogeneity of SV for size may reflect their different functions. In investigations conducted on the electric organ of the skate [6] heterogeneity of SV with respect to biochemical (functional) properties and correlation between the latter and the size of the vesicles also have been demonstrated.

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CARDIOMYOCYTE ULTRASTRUCTURE IN THE PERIFOCAL ZONE OF AN EXPERIMENTAL MYOCARDIAL INFARCT IN RATS TREATED WITH THE HEXAPEPTIDE DALARGIN

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Intensive research is in progress at the present time to find drugs capable of reducing the size of a zone of myocardial infarction [1, 2, 10, 14]. Much attention is being paid to the perifocal zone of the myocardial infarct as the target object for therapeutic activity aimed at restricting the spread of necrosis among cardiomyocytes [6, 9]. Enkephalins are known to have an antistressor action and to prevent release of various hormones that participate in the catabolic phase of stress, as well as preventing the peripheral action of catecholamines [4, 7]. The authors showed previously that administration of the hexapeptide dalargin to rats after coronary artery occlusion leads to a decrease in size of the infarct [8].

The aim of this investigation was to determine the most effective dose of dalargin in experimental myocardial infarction and also to assess its effect on cardiomyocytes of the perifocal zone surrounding the infarct at the ultrastructural level.

EXPERIMENTAL METHOD

Myocardial infarction was induced in 76 noninbred albino rats weighing 180-200 g by ligation of the descending branch of the left coronary artery in its upper third. The rats of the experimental groups received dalargin by intraperitoneal injection in doses of 10, 50, 100, 500, and 1000 μg/kg and rats of the control group received physiological saline 1 h after ligation. The rats were decapitated 24 h after coronary occlusion and the size of the zone of infarction in the left ventricle was determined by demonstration of phosphorylase activity followed by planimetry [15], and also by the macroscopic reaction with nitro-BT followed by gravimetry [3]. Specimens of myocardium for electron microscopy were excised from the region around the infarct, fixed, and embedded in Araldite in the usual way. Some

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TABLE 1. Quantitative Analysis of Electron Micrographs of Perifocal Zone of Left Ventricular Myocardial Infarct in Rat 24 h after Coronary Arterial Ligation (M ± m)

Parameters	Control	MI	MI + dalargin
Number of mitochondria in one electron micrograph	27,7±1,05	17,67±0,79	26,75±0,96
p_1		<0,001	>0,05
p_2			<0,001
Number of cristae per mitochondrion	12,45±0,25	9,97±0,32	9,47±0,39
p_1		<0,01	<0,01
p_2			>0,05
Average area of one mitochondrion, μ^2	0,46±0,01	0,57±0,01	0,50±0,03
p_1		<0,01	>0,05
p_2			<0,05
Average area of mitochondria in one electron micrograph, μ^2	12,83±0,54	9,85±0,38	12,89±0,69
p_1		<0,001	>0,05
p_2			<0,001
Number of cristae per electron micrograph	341±11,5	174,8±8,1	251,2±11,2
p_1		<0,001	<0,01
p_2			<0,001
MEEC, %	100	39,69±2,66	76,68±6,9
p_2			<0,01

Legend. MI) Myocardial infarction; control — parameters for myocardium of intact rats; p_1) significance compared with control; p_2) compared with untreated rats.

specimens were incubated with colloidal lanthanum during fixation [12]. Ultrathin sections, cut on the Ultrotome-5 (LKB, Sweden), were stained in an Ultrastainer (LKB). The mitochondrial energy efficiency coefficient (MEEC) [9], which is the ratio of the product of the mean total number of cristae and the total area of the mitochondria in one electron micrograph in pathology to the same parameters of the normal heart, expressed as a percentage, was determined on the electron micrographs under a magnification of 10,000. Altogether 108 electron micrographs were studied. The significance of differences was determined by Student's test.

EXPERIMENTAL RESULTS

The infarct in groups of animals receiving dalargin, measured 24 h after ligation of the coronary artery, was smaller than in the control. A significant decrease in size of the infarct was recorded in groups treated with dalargin in doses of 50 and 100 μg/kg (45.65 ± 2.29%; $p < 0.01$ and 49.57 ± 1.61%; $p < 0.05$ respectively). With a change in the dose of the drug in either the downward or the upward direction this effect was progressively reduced (53.97 ± 2.15% with a dose of 10 μg/ml, 52.34 ± 2.3% with a dose of 500 μg/ml; 52.24 ± 2.32% with a dose of 1000 μg/ml), and the difference from the control group (55.76 ± 1.68%) ceased to be significant ($p > 0.05$), in agreement with data in the literature on the character of action of regulatory peptides [11]. The electron-microscopic study of the myocardium in the zone surrounding the infarct was carried out in a group of animals receiving dalargin in a dose of 50 μg/kg, for administration of dalargin in this dose gave the most marked effect. Ultrastructural changes in the perifocal myocardium in rats of the control group corresponded to those described previously [6]: infiltration of cardiomyocytes by lipids, the almost total disappearance of glycogen, moderate destruction of the organelles, slight aggregation of nuclear chromatin (Fig. 1a, b). A considerable increase in the glycogen content, less marked infiltration of the cells with lipids, and fewer injured mitochondria and myofibrils were observed (Fig. 1c, d) in the cardiomyocytes in the zone surrounding the infarct in rats treated with dalargin. Particles of colloidal lanthanum began to penetrate into the sarcoplasm of the cardiomyocytes only after quite large defects had formed in the sarcolemma, and this moment coincided with the beginning of irreversible ischemic damage to the cardiomyocytes [13]. Virtually all cardiomyocytes in the perifocal myocardium in rats of the control group contained particles of colloidal lanthanum in their sarcoplasm, although no particles penetrated inside the mitochondria, evidence of the irreversible character of ischemic damage to these cells (Fig. 2a). In rats treated with dalargin only solitary cells of the perifocal zone contained lanthanum particles in their sarcoplasm. The sarcolemma of most cardiomyocytes was impenetrable for the tracer (Fig. 2b). Analysis of the morphometric

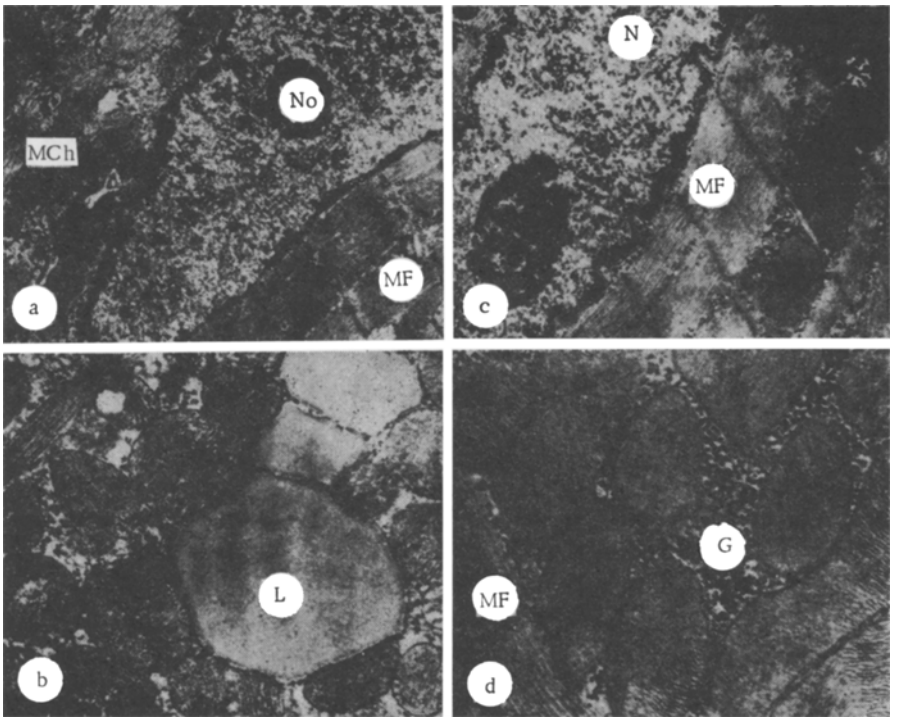

Fig. 1. Cardiomyocytes of perifocal zone surrounding myocardial infarct in rat 24 h after coronary arterial ligation. a, b) Untreated rats; c, d) rats treated with dalargin; N) nucleus, No) nucleolus; MCh) mitochondria; MF) myofibrils; G) glycogen; L) lipid droplets. Magnification: a, c) 7000, b, d) 10,000×.

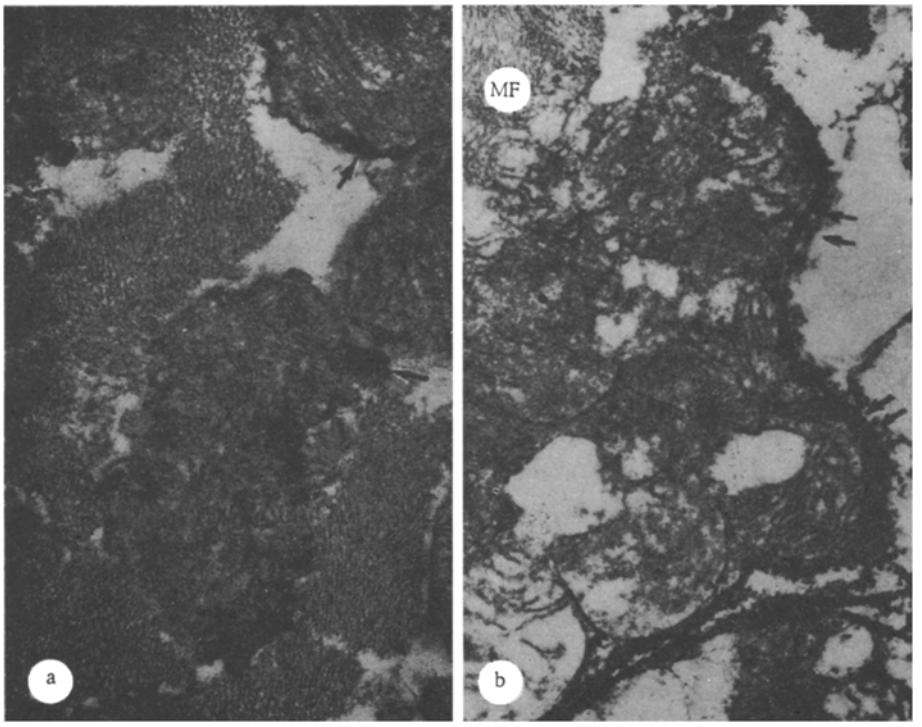

Fig. 2. Cardiomyocytes of perifocal zone of infarct in rat 24 h after coronary arterial ligation; incubation with colloidal lanthanum. a) Untreated rats, colloidal lanthanum particles (arrows) around mitochondria; b) rats treated with dalargin, colloidal lanthanum particles (arrows) on outer aspect of sarcolemma. MF) Myofibrils. 20,000×.

parameters for the perifocal zone showed that the number of mitochondria and the average number of cristae per mitochondrion were significantly reduced in the untreated rats. The average area of mitochondria was significantly greater than in the control. All these changes led to a decrease in MEEC to 39.69% of its value for the normal myocardium. In rats treated with dalargin this parameter was significantly higher, namely 76.68% (Table 1). The number of mitochondria in this group was the same as in normal myocardium, but the mean number of cristae in them as significantly less, and it was this which caused the decrease in MEEC.

It can thus be concluded from these results that administration of dalargin in the early stages after coronary arterial ligation in rats has a protective action on the cardiomyocytes in the zone surrounding the infarct. As a result most cells of this zone remain viable and do not subsequently undergo necrosis. This is responsible for the effect, described by the authors previously [8], of reduction in size of the infarct zone under the influence of dalargin.

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